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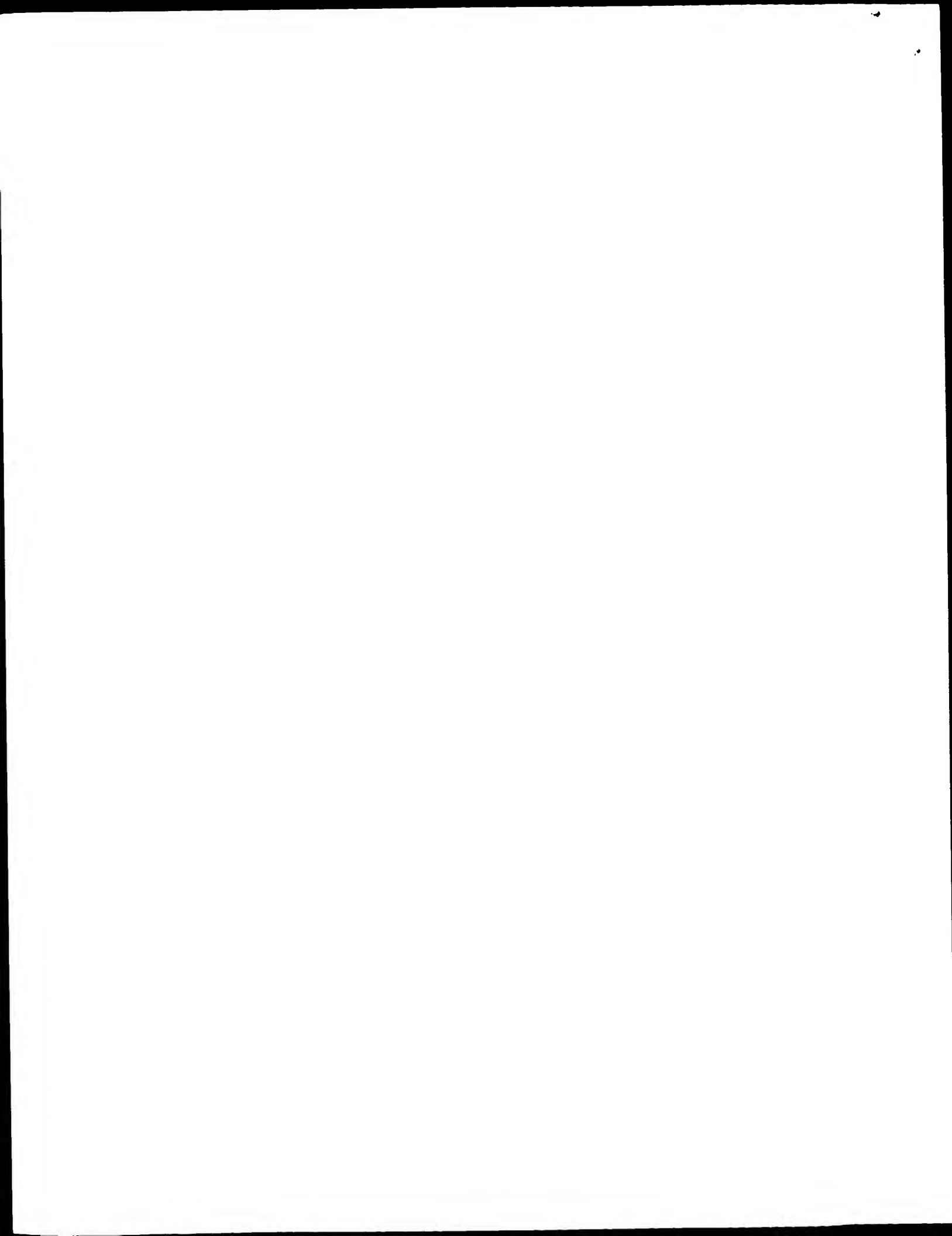
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<p>(21) International Application Number: PCT/US98/06774 (22) International Filing Date: 6 April 1998 (06.04.98) (30) Priority Data: 08/833,488 7 April 1997 (07.04.97) US (71) Applicant: HESKA CORPORATION [US/US]; 1825 Sharp Point Drive, Fort Collins, CO 80525 (US). (72) Inventors: FRANK, Glenn, Robert; 10317 North County Road 13, Wellington, CO 80549 (US). RUSHLOW, Keith, E.; 1600 Dogwood Court, Fort Collins, CO 80525 (US). (74) Agents: HANLEY, Elizabeth, A. et al.; Lahive & Cockfield, LLP, 28 State Street, Boston, MA 02109 (US).</p>		<p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report.</i></p>
<p>(54) Title: METHOD TO DETECT CANINE IgE (57) Abstract The present invention includes a method to detect canine IgE using a canine Fc epsilon receptor (FcεR) to detect canine IgE antibodies in a biological sample from a canid. The present invention also relates to kits to perform such methods.</p>		



METHOD TO DETECT CANINE IgE

Field of the Invention

The present invention relates to a novel method to detect canine epsilon immunoglobulin (IgE). The present invention also includes novel kits to detect canine
5 IgE as well as methods to produce the detection reagent.

Background of the Invention

Diagnosis of disease and determination of treatment efficacy are important tools in medicine. In particular, detection of IgE production in an animal can be indicative of disease. Such diseases include, for example, allergy, atopic disease, hyper IgE
10 syndrome, internal parasite infections and B cell neoplasia. In addition, detection of IgE production in an animal following a treatment is indicative of the efficacy of the treatment, such as when using treatments intended to disrupt IgE production.

Until the discovery of the present invention, detection of IgE in samples obtained from non-human animals has been hindered by the absence of suitable reagents for
15 detection of IgE. Various compounds have been used to detect IgE in IgE-containing compositions. In particular, antibodies that bind selectively to epsilon idiotype antibodies (i.e., anti-IgE antibodies) have been used to detect IgE. These anti-IgE antibodies, however, can cross-react with other antibody idiotypes, such as gamma isotype antibodies. The discovery of the present invention includes the use of a canine
20 Fc epsilon receptor (Fc_εR) molecule to detect the presence of IgE in a putative IgE-containing composition. Canine high affinity Fc_εR consists of three protein chains, alpha, beta and gamma. Hayashi et al. have disclosed the nucleic acid sequence for the alpha chain (GenBank Accession No. D16413, submitted June 8, 1993). A canine Fc_εR molecule provides an advantage over, for example anti-IgE antibodies, to detect IgE
25 because a canine Fc_εR molecule can bind to a canine IgE with more specificity (i.e., less idiotype cross-reactivity) and more sensitivity (i.e., affinity) than anti-IgE binding antibodies.

Thus, methods and kits are needed in the art that will provide specific detection of canine IgE using canine Fc_εR.

Summary of the Invention

The present invention includes detection methods and kits that detect canine IgE.

One embodiment of the present invention is a method to detect canine IgE comprising: (a) contacting an isolated canine Fc_ϵ receptor ($Fc_\epsilon R$) molecule with a putative canine IgE-containing composition under conditions suitable for formation of a $Fc_\epsilon R$ molecule:IgE complex; and (b) determining the presence of IgE by detecting the $Fc_\epsilon R$ molecule:IgE complex, the presence of the $Fc_\epsilon R$ molecule:IgE complex indicating the presence of IgE. In particular, the canine $Fc_\epsilon R$ molecule comprises at least a portion of a $Fc_\epsilon R$ alpha chain that binds to canine IgE.

Another embodiment of the present invention is a method to detect canine flea allergy dermatitis comprising: (a) immobilizing a flea allergen on a substrate; (b) contacting the flea allergen with a putative canine IgE-containing composition under conditions suitable for formation of an antigen:IgE complex bound to the substrate; (c) removing non-bound material from the substrate under conditions that retain antigen:IgE complex binding to the substrate; and (d) detecting the presence of the antigen:IgE complex by contacting antigen:IgE complex with a canine $Fc_\epsilon R$ molecule. In particular, the flea allergen is a flea saliva antigen.

The present invention also includes a kit for performing methods of the present invention. One embodiment is a kit for detecting IgE comprising a canine Fc_ϵ receptor ($Fc_\epsilon R$) molecule and a means for detecting canine IgE. Another embodiment is a kit for detecting flea allergy dermatitis comprising a canine Fc_ϵ receptor ($Fc_\epsilon R$) molecule and a flea allergen.

Detailed Description of the Invention

The present invention relates to the discovery that purified high affinity canine Fc_ϵ epsilon receptor (i.e., $Fc_\epsilon RI$; referred to herein as $Fc_\epsilon R$) can be used in canine epsilon immunoglobulin (referred to herein as IgE or IgE antibody)-based detection (e.g., diagnostic, screening) methods and kits. The use of canine $Fc_\epsilon R$ in diagnostic methods and kits is unexpected because the use of canine $Fc_\epsilon R$ avoids complications presented by use of antibodies that bind to IgE (i.e., anti-IgE antibodies). Such complications include, for example, non-specific binding of anti-IgE antibodies to other classes of immunoglobulin such as gamma immunoglobulin (i.e., IgG).

One embodiment of the present invention is a method to detect a canine IgE using an isolated canine $Fc_\epsilon R$ molecule. It is to be noted that the term "a" entity or "an" entity refers to one or more of that entity; for example, a protein refers to one or more proteins or at least one protein. As such, the terms "a" (or "an"), "one or more" and "at least one" can be used interchangeably herein. It is also to be noted that the terms "comprising", "including", and "having" can be used interchangeably. Furthermore, a compound "selected from the group consisting of" refers to one or more of the compounds in the list that follows, including mixtures (i.e., combinations) of two or more of the compounds.

10 According to the present invention, an isolated, or biologically pure, $Fc_\epsilon R$ molecule, is a molecule that has been removed from its natural milieu. As such, "isolated" and "biologically pure" do not necessarily reflect the extent to which the molecule has been purified. An isolated canine $Fc_\epsilon R$ molecule of the present invention can be obtained from its natural source (e.g., from a canine mast cell), can be produced using recombinant DNA technology or can be produced by chemical synthesis.

A $Fc_\epsilon R$ molecule (also referred to herein as $Fc_\epsilon R$ or $Fc_\epsilon R$ protein) of the present invention can be a full-length protein, a portion of a full-length protein or any homolog of such a protein, wherein the $Fc_\epsilon R$ molecule is capable of binding specifically to IgE. As used herein, a protein can be a polypeptide or a peptide. A $Fc_\epsilon R$ molecule of the present invention can comprise a complete $Fc_\epsilon R$ (i.e., alpha, beta and gamma $Fc_\epsilon R$ chains), an alpha $Fc_\epsilon R$ chain (also referred to herein as $Fc_\epsilon R$ α chain) or portions thereof. Preferably, a $Fc_\epsilon R$ molecule comprises at least a portion of a $Fc_\epsilon R$ α chain that binds to IgE, i.e., that is capable of forming an immunocomplex with an IgE constant region.

An isolated canine $Fc_\epsilon R$ molecule of the present invention, including a homolog, 25 can be identified in a straight-forward manner by the $Fc_\epsilon R$ molecule's ability to form an immunocomplex with a canine IgE. Examples of $Fc_\epsilon R$ homologs include $Fc_\epsilon R$ proteins in which amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide), inserted, inverted, substituted and/or derivatized (e.g., by glycosylation, phosphorylation, acetylation, myristoylation, prenylation, palmitoylation, amidation and/or addition of glycerophosphatidyl inositol) such that the homolog includes at least 30 one epitope capable of forming an immunocomplex with an IgE.

Fc_εR homologs can be the result of natural allelic variation or natural mutation. Fc_εR homologs of the present invention can also be produced using techniques known in the art including, but not limited to, direct modifications to the protein or modifications to the gene encoding the protein using, for example, classic or recombinant DNA techniques to effect random or targeted mutagenesis.

According to the present invention, a preferred canine Fc_εR α chain of the present invention is encoded by at least a portion of the nucleic acid sequence of the coding strand of a cDNA encoding a full-length Fc_εR α chain protein represented herein as SEQ ID NO:19, the portion at least encoding the IgE binding site of the Fc_εR α chain protein. Other suitable canine Fc_εR α chains useful in the present invention include those described herein in the Examples section. The double-stranded nucleic acid molecule including both the coding strand having SEQ ID NO:19 and the complementary non-coding strand (the nucleic acid sequence of which can be readily determined by one skilled in the art and is shown herein as SEQ ID NO:21) is referred to herein as Fc_εR nucleic acid molecule ncFc_εRα4₉₉₁. Translation of SEQ ID NO:19 suggests that nucleic acid molecule ncFc_εRα4₉₉₁ encodes a full-length Fc_εR α chain protein of about 253 amino acids, referred to herein as PcFc_εRα4₂₅₃, represented by SEQ ID NO:20, assuming an open reading frame having an initiation (start) codon spanning from about nucleotide 35 through about nucleotide 37 of SEQ ID NO:19 and the termination codon spans from about nucleotide 793 through about nucleotide 795 of SEQ ID NO:19. The coding region encoding PcFc_εRα4₂₅₃, excluding the stop codon, is represented by nucleic acid molecule ncFc_εRα4₇₃₉, having a coding strand with the nucleic acid sequence represented by SEQ ID NO:22 and a complementary strand with nucleic acid sequence SEQ ID NO:23. SEQ ID NO:19 encodes a signal peptide spanning from about amino acid 1 through about amino acid 24, as well as a mature protein of about 229 amino acids, denoted herein as PcFc_εRα4₂₂₉, the amino acid sequence of which is represented herein as SEQ ID NO:24. The nucleic acid molecule encoding the apparent mature protein is referred to as ncFc_εRα4₆₈₇, the nucleic acid sequence of the coding strand of which is denoted herein as SEQ ID NO:30. SEQ ID NO:19 also encodes a hydrophobic transmembrane domain which extends from about amino acid 172 to about amino acid 228 of SEQ ID NO:24. Knowledge of these nucleic

acid and amino acid sequences allows one skilled in the art to make modifications to the respective nucleic acid molecules and proteins to, for example, develop a canine $Fc_\epsilon R \alpha$ chain protein with increased solubility and/or a truncated protein capable of detecting canine IgE, e.g., $PcFc_\epsilon R\alpha_{4_{197}}$, spanning from about amino acid 1 to about amino acid 197 of SEQ ID NO:20, and having SEQ ID NO:28; or $PcFc_\epsilon R\alpha_{4_{173}}$, spanning from about amino acid 25 to about amino acid 197 of SEQ ID NO:20, and having SEQ ID NO:31.

Preferred $Fc_\epsilon R$ molecules include $PcFc_\epsilon R\alpha_{4_{253}}$, $PcFc_\epsilon R\alpha_{4_{229}}$, $PcFc_\epsilon R\alpha_{4_{197}}$, $PcFc_\epsilon R\alpha_{4_{173}}$ and allelic variants thereof, as well as $PcFc_\epsilon R\alpha_{1_{197}}$, $PcFc_\epsilon R\alpha_{2_{197}}$, $PcFc_\epsilon R\alpha_{3_{199}}$ (which are disclosed in the Examples section) and allelic variants thereof.

Preferred nucleic acid molecules to encode a $Fc_\epsilon R$ molecules include $ncFc_\epsilon R\alpha_{4_{591}}$, $ncFc_\epsilon R\alpha_{4_{687}}$, $ncFc_\epsilon R\alpha_{4_{991}}$, $ncFc_\epsilon R\alpha_{4_{759}}$ and allelic variants thereof, as well as $ncFc_\epsilon R\alpha_{1_{609}}$, $ncFc_\epsilon R\alpha_{1_{591}}$, $ncFc_\epsilon R\alpha_{2_{609}}$, $ncFc_\epsilon R\alpha_{2_{591}}$, $ncFc_\epsilon R\alpha_{3_{617}}$, $ncFc_\epsilon R\alpha_{3_{597}}$ (which are disclosed in the Examples section) and allelic variants thereof. A preferred nucleic acid sequence encoding a canine $Fc_\epsilon R$ molecule includes SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:19, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:30, and/or a nucleic acid molecule comprising an allelic variant of a nucleic acid molecule comprising any of said nucleic acid sequences.

An isolated canine $Fc_\epsilon R$ molecule protein of the present invention can be produced by culturing a cell capable of expressing the protein under conditions effective to produce the protein, and recovering the protein. A preferred cell to culture is a recombinant cell that is capable of expressing the protein, the recombinant cell being produced by transforming a host cell with one or more nucleic acid molecules of the present invention. Transformation of a nucleic acid molecule into a cell can be accomplished by any method by which a nucleic acid molecule can be inserted into the cell. Transformation techniques include, but are not limited to, transfection, electroporation, microinjection, lipofection, adsorption, and protoplast fusion. A recombinant cell may remain unicellular or may grow into a tissue, organ or a multicellular organism. Transformed nucleic acid molecules of the present invention can remain extrachromosomal or can integrate into one or more sites within a chromosome of the transformed (i.e., recombinant) cell in such a manner that their

ability to be expressed is retained. Suitable and preferred nucleic acid molecules with which to transform a cell are as disclosed herein for suitable and preferred Fc_εR nucleic acid molecules per se. Particularly preferred nucleic acid molecules to include in recombinant cells of the present invention include ncFc_εRα1₆₀₉, ncFc_εRα1₅₉₁,
 5 ncFc_εRα2₆₀₉, ncFc_εRα2₅₉₁, ncFc_εRα3₆₁₇, ncFc_εRα3₅₉₇, ncFc_εRα4₅₉₁, ncFc_εRα4₆₈₇,
 ncFc_εRα4₉₉₁ and/or ncFc_εRα4₇₅₉.

Suitable host cells to transform include any cell that can be transformed with a nucleic acid molecule of the present invention. Host cells can be either untransformed cells or cells that are already transformed with at least one nucleic acid molecule. Host
 10 cells of the present invention either can be endogenously (i.e., naturally) capable of producing a canine Fc_εR molecule protein of the present invention or can be capable of producing such proteins after being transformed with at least one nucleic acid molecule of the present invention. Host cells of the present invention can be any cell capable of producing at least one protein of the present invention, and include bacterial, fungal
 15 (including yeast), parasite (including protozoa and ectoparasite), insect, other animal and plant cells.

Preferably, a recombinant cell is transfected with a recombinant molecule of the present invention. A recombinant molecule of the present invention includes at least one of any nucleic acid molecules heretofore described operatively linked to at least one of
 20 any transcription control sequence capable of effectively regulating expression of the nucleic acid molecule(s) in the cell to be transformed, examples of which are disclosed herein. Particularly preferred recombinant molecules include pVL-ncFc_εRα4₅₉₁, pVL-ncFc_εRα1₆₀₉, pVL-ncFc_εRα2₆₀₉, and pVL-ncFc_εRα3₆₁₇. Details regarding the production of Fc_εR molecule nucleic acid molecule-containing recombinant molecules are disclosed
 25 herein. Particularly preferred recombinant cells of the present invention include *S. frugiperda*:pVL-ncFc_εRα4₅₉₁, *Trichoplusia ni*:BV-ncFc_εRα4₅₉₁, *S. frugiperda*:pVL-ncFc_εRα1₆₀₉, *S. frugiperda*:pVL-ncFc_εRα2₆₀₉, *S. frugiperda*:pVL-ncFc_εRα3₆₀₈,
Trichoplusia ni:BV-ncFc_εRα1₆₀₉, *Trichoplusia ni*:BV-ncFc_εRα2₆₀₉, and *Trichoplusia ni*:BV-ncFc_εRα3₆₁₇.

30 A Fc_εR molecule of the present invention can include chimeric molecules comprising a portion of a Fc_εR molecule that binds to an IgE and a second molecule that

enables the chimeric molecule to be bound to a substrate in such a manner that the $Fc_\epsilon R$ portion binds to IgE in essentially the same manner as a $Fc_\epsilon R$ molecule that is not bound to a substrate. An example of a suitable second molecule includes a portion of an immunoglobulin molecule.

- 5 A canine $Fc_\epsilon R$ molecule of the present invention can be contained in a formulation, herein referred to as a $Fc_\epsilon R$ formulation. For example, a canine $Fc_\epsilon R$ molecule can be combined with a buffer in which the $Fc_\epsilon R$ is solubilized, and/or with a carrier. Suitable buffers and carriers are known to those skilled in the art. Examples of suitable buffers include any buffer in which a $Fc_\epsilon R$ can function to selectively bind to
- 10 IgE, such as, but not limited to, phosphate buffered saline, water, saline, phosphate buffer, bicarbonate buffer, HEPES buffer (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid buffered saline), TES buffer (Tris-EDTA buffered saline), Tris buffer and TAE buffer (Tris-acetate-EDTA). Examples of carriers include, but are not limited to, polymeric matrices, toxoids, and serum albumins, such as bovine serum
- 15 albumin. Carriers can be combined with $Fc_\epsilon R$ or conjugated (i.e., attached) to $Fc_\epsilon R$ in such a manner as to not substantially interfere with the ability of the $Fc_\epsilon R$ to selectively bind to IgE.

- A canine $Fc_\epsilon R$ molecule of the present invention can be bound to the surface of a cell expressing the $Fc_\epsilon R$. A preferred $Fc_\epsilon R$ -bearing cell includes a recombinant cell
- 20 expressing a nucleic acid molecule encoding a canine $Fc_\epsilon R$ alpha chain of the present invention. A more preferred recombinant cell of the present invention expresses a nucleic acid molecule that encodes at least one of the following proteins: $PcFc_\epsilon R\alpha 1_{197}$, $PcFc_\epsilon R\alpha 2_{197}$, $PcFc_\epsilon R\alpha 3_{199}$, $PcFc_\epsilon R\alpha 4_{253}$, $PcFc_\epsilon R\alpha 4_{229}$, $PcFc_\epsilon R\alpha 4_{197}$ and $PcFc_\epsilon R\alpha 4_{173}$. An even more preferred recombinant cell expresses a nucleic acid molecule including
- 25 $ncFc_\epsilon R\alpha 1_{609}$, $ncFc_\epsilon R\alpha 1_{591}$, $ncFc_\epsilon R\alpha 2_{609}$, $ncFc_\epsilon R\alpha 2_{591}$, $ncFc_\epsilon R\alpha 3_{617}$, $ncFc_\epsilon R\alpha 3_{597}$, $ncFc_\epsilon R\alpha 4_{591}$, $ncFc_\epsilon R\alpha 4_{687}$, $ncFc_\epsilon R\alpha 4_{991}$ and $ncFc_\epsilon R\alpha 4_{759}$, or allelic variants thereof, with a recombinant cell expressing a nucleic acid molecule comprising a nucleic acid sequence SEQ ID NO:3, SEQ ID NO:8, SEQ ID NO:13 or SEQ ID NO:27, or a nucleic acid molecule comprising an allelic variant of a nucleic acid molecule comprising SEQ
- 30 ID NO:3, SEQ ID NO:8, SEQ ID NO:13 or SEQ ID NO:27, being even more preferred.

In addition, a $Fc_\epsilon R$ formulation of the present invention can include not only a $Fc_\epsilon R$ but also one or more additional antigens or antibodies useful in detecting IgE. As used herein, an antigen refers to any molecule capable of being selectively bound by an antibody. As used herein, specific binding of a first molecule to a second molecule refers to the ability of the first molecule to preferentially bind to (e.g., have higher affinity higher avidity for) the second molecule when compared to the ability of a first molecule to bind to a third molecule. The first molecule need not necessarily be the natural ligand of the second molecule. Examples of antibodies used in the present invention include, but are not limited to, antibodies that bind selectively to the constant region of an IgE heavy chain (i.e., anti-IgE isotype antibodies) or antibodies that bind selectively to an IgE having a specific antigen specificity (i.e., anti-IgE idiotype antibodies). Examples of antigens used in the present invention include any antigen known to induce the production of IgE. Preferred antigens include allergens and parasite antigens. Allergens of the present invention are preferably derived from fungi, trees, weeds, shrubs, grasses, wheat, corn, soybeans, rice, eggs, milk, cheese, bovines (or cattle), poultry, swine, sheep, yeast, fleas, flies, mosquitos, mites, midges, biting gnats, lice, bees, wasps, ants, true bugs or ticks. A suitable flea allergen includes an allergen derived from a flea, and in particular a flea saliva antigen. Preferred flea saliva antigens include antigens such as those disclosed in PCT Patent Publication No. WO 96/11271, published April 18, 1996, by Frank et al. (this publication is incorporated by reference herein in its entirety), U.S. Patent Application Serial Nos. 08/319,590 (filed Oct. 7, 1994), 08/487,001 (filed June 7, 1995), 08/487,608 (filed June 7, 1995) and 08/630,822 (filed April 10, 1996), with flea saliva products and flea saliva proteins being particularly preferred. According to the present invention, a flea saliva protein includes a protein produced by recombinant DNA methods, as well as proteins isolated by other methods disclosed in PCT Patent Publication No. WO 96/11271, U.S. Patent Application Serial Nos. 08/319,590 (filed Oct. 7, 1994), 08/487,001 (filed June 7, 1995), 08/487,608 (filed June 7, 1995) and 08/630,822 (filed April 10, 1996).

Preferred general allergens include those derived from grass, Meadow Fescue, Curly Dock, plantain, Mexican Firebush, Lamb's Quarters, pigweed, ragweed, sage, elm, cocklebur, Box Elder, walnut, cottonwood, ash, birch, cedar, oak, mulberry, cockroach,

- Dermataphagoides*, *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Helminthosporium*, *Mucor*, *Penicillium*, *Pullularia*, *Rhizopus* and/or *Tricophyton*. More preferred general allergens include those derived from Johnson Grass, Kentucky Blue Grass, Meadow Fescue, Orchard Grass, Perennial Rye Grass, Redtop Grass, Timothy
- 5 Grass, Bermuda Grass, Brome Grass, Curly Dock, English Plantain, Mexican Firebush, Lamb's Quarters, Rough Pigweed Short Ragweed, Wormwood Sage, American Elm, Common Cocklebur, Box Elder, Black Walnut, Eastern Cottonwood, Green Ash, River Birch, Red Cedar, Red Oak, Red Mulberry, Cockroach, *Dermataphagoides farinae*, *Alternaria alternata*, *Aspergillus fumigatus*, *Cladosporium herbarum*, *Fusarium*
- 10 *vasinfectum*, *Helminthosporium sativum*, *Mucor recemosus*, *Penicillium notatum*, *Pullularia pullulans*, *Rhizopus nigricans* and/or *Tricophyton* spp. Preferred parasite antigens include, but are not limited to, helminth antigens, in particular heartworm antigens, such as Di33 (described in U.S. Patent Application Serial No. 08/715,628, filed September 18, 1996, to Grieve et al.; this publication is incorporated by reference herein
- 15 in its entirety). The term "derived from" refers to a natural allergen of such plants or organisms (i.e., an allergen directly isolated from such plants or organisms), as well as non-natural allergens of such plants or organisms that possess at least one epitope capable of eliciting an immune response against an allergen (e.g., produced using recombinant DNA technology or by chemical synthesis).
- 20 The present invention also includes canine Fc_εR mimetopes and use thereof to detect IgE. In accordance with the present invention, a "mimetope" refers to any compound that is able to mimic the ability of a canine Fc_εR molecule to bind to canine IgE. A mimetope can be a peptide that has been modified to decrease its susceptibility to degradation but that still retains IgE-binding activity. Other examples of mimetopes
- 25 include, but are not limited to, carbohydrate-based compounds, lipid-based compounds, nucleic acid-based compounds, natural organic compounds, synthetically derived organic compounds, anti-idiotypic antibodies and/or catalytic antibodies, or fragments thereof. A mimetope can be obtained by, for example, screening libraries of synthetic compounds for compounds capable of binding to IgE. A mimetope can also be obtained
- 30 by, for example, rational drug design. In a rational drug design procedure, the three-dimensional structure of a compound of the present invention can be analyzed by, for

example, nuclear magnetic resonance (NMR) or x-ray crystallography. The three-dimensional structure can then be used to predict structures of potential mimetopes by, for example, computer modeling. The predicted mimetope structures can then be produced by, for example, chemical synthesis, recombinant DNA technology, or by
5 isolating a mimetope from a natural source. Specific examples of $Fc_\epsilon R$ mimetopes include anti-idiotypic antibodies, oligonucleotides produced using Selex® technology, peptides identified by random screening of peptide libraries and proteins identified by phage display technology.

One embodiment of the present invention is a method to detect canine IgE which
10 includes the steps of: (a) contacting an isolated canine Fc_ϵ receptor ($Fc_\epsilon R$) molecule with a putative canine IgE-containing composition under conditions suitable for formation of a $Fc_\epsilon R$ molecule:IgE complex; and (b) detecting levels of IgE by detecting said $Fc_\epsilon R$ molecule:IgE complex. Presence of such a $Fc_\epsilon R$ molecule:IgE complex indicates that the canine is producing IgE. The present method can further include the step of
15 determining whether a canine IgE complexed with a canine $Fc_\epsilon R$ molecule is heat labile. Certain classes of IgE are heat labile when incubated at about 56°C for about 4 hours. Without being bound by theory, Applicants believe that heat labile forms of IgE bind to certain allergens and non-heat labile forms of IgE bind to other types of allergens. As such, detection of heat labile IgE compared with non-heat labile IgE can be used to
20 discriminate between allergen sensitivities. For example, Applicants believe that canine IgE antibodies that bind to certain flea allergens and heartworm allergens are heat labile while canine IgE antibodies that bind to certain plant allergens are not heat labile. Thus, the presence of non-heat labile IgE can indicate that an animal is sensitive to certain plant allergens but not to certain flea or heartworm allergens. Moreover, Applicants
25 believe that identification of heat labile IgE and non-heat labile IgE using a canine $Fc_\epsilon R$ suggests the presence of different sub-populations of IgE that may or may not have substantially similar structures to known IgE. As such, a $Fc_\epsilon R$ molecule of the present invention may be useful for detecting molecules bound by the $Fc_\epsilon R$ molecule that are not identical to a known IgE.

As used herein, canine refers to any member of the dog family, including domestic dogs, wild dogs and zoo dogs. Examples of dogs include, but are not limited to, domestic dogs, wild dogs, foxes, wolves, jackals and coyotes.

As used herein, the term "contacting" refers to combining or mixing, in this case
5 a putative IgE-containing composition with a canine $Fc_\epsilon R$ molecule. Formation of a complex between a canine $Fc_\epsilon R$ and a canine IgE refers to the ability of the $Fc_\epsilon R$ to selectively bind to the IgE in order to form a stable complex that can be measured (i.e., detected). As used herein, the term selectively binds to an IgE refers to the ability of a $Fc_\epsilon R$ of the present invention to preferentially bind to IgE, without being able to
10 substantially bind to other antibody isotypes. Binding between a $Fc_\epsilon R$ and an IgE is effected under conditions suitable to form a complex; such conditions (e.g., appropriate concentrations, buffers, temperatures, reaction times) as well as methods to optimize such conditions are known to those skilled in the art, and examples are disclosed herein. Examples of complex formation conditions are also disclosed in, for example, in
15 Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press, 1989; the reference Sambrook et al., *ibid.*, is incorporated by reference herein in its entirety.

As used herein, the term "detecting complex formation" refers to determining if any complex is formed, i.e., assaying for the presence (i.e., existence) of a complex. If
20 complexes are formed, the amount of complexes formed can, but need not be, determined. Complex formation, or selective binding, between canine $Fc_\epsilon R$ and canine IgE in the composition can be measured (i.e., detected, determined) using a variety of methods standard in the art (see, for example, Sambrook et al. *ibid.*), examples of which are disclosed herein.

25 In one embodiment, a putative canine IgE-containing composition of the present method includes a biological sample from a canine. A suitable biological sample includes, but is not limited to, a bodily fluid composition or a cellular composition. A bodily fluid refers to any fluid that can be collected (i.e., obtained) from an animal, examples of which include, but are not limited to, blood, serum, plasma, urine, tears,
30 aqueous humor, central nervous system fluid (CNF), saliva, lymph, nasal secretions, milk and feces. Such a composition of the present method can, but need not be,

pretreated to remove at least some of the non-IgE isotypes of immunoglobulin and/or other proteins, such as albumin, present in the fluid. Such removal can include, but is not limited to, contacting the bodily fluid with a material, such as Protein G, to remove IgG antibodies and/or affinity purifying IgE antibodies from other components of the body fluid by exposing the fluid to, for example, Concanavalin A. In another embodiment, a composition includes collected bodily fluid that is pretreated to concentrate immunoglobulin contained in the fluid. For example, immunoglobulin contained in a bodily fluid can be precipitated from other proteins using ammonium sulfate. A preferred composition of the present method is serum.

10 In another embodiment, a composition of the present method includes an IgE-producing cell. Such a cell can have IgE bound to the surface of the cell and/or can secrete IgE. Examples of such cells include basophil cells and myeloma cells. IgE can be bound to the surface of a cell, for example by being either bound directly to the membrane of a cells or bound to a molecule (e.g., an antigen) bound to the surface of the cell.

15 A complex can be detected in a variety of ways including, but not limited to, use of one or more of the following assays: an enzyme-linked immunoassay, a radioimmunoassay, a fluorescence immunoassay, a chemiluminescent assay, a lateral flow assay, an agglutination assay, a particulate-based assay (e.g., using particulates such as, but not limited to, magnetic particles or plastic polymers, such as latex or polystyrene beads), an immunoprecipitation assay, a BioCore™ assay (e.g., using colloidal gold) and an immunoblotting assay (e.g., a western blot). Such assays are well known to those skilled in the art. Assays can be used to give qualitative or quantitative results depending on how they are used. Some assays, such as agglutination, particulate separation, and immunoprecipitation, can be observed visually (e.g., either by eye or by a machine, such as a densitometer or spectrophotometer) without the need for a detectable marker. In other assays, conjugation (i.e., attachment) of a detectable marker to the Fc_εR or to a reagent that selectively binds to the Fc_εR or to the IgE being detected (described in more detail below) aids in detecting complex formation. Examples of detectable markers include, but are not limited to, a radioactive label, a fluorescent label, a chemiluminescent label, a chromophoric label or a ligand. A ligand refers to a molecule

that binds selectively to another molecule. Preferred detectable markers include, but are not limited to, fluorescein, a radioisotope, a phosphatase (e.g., alkaline phosphatase), biotin, avidin, a peroxidase (e.g., horseradish peroxidase) and biotin-related compounds or avidin-related compounds (e.g., streptavidin or ImmunoPure® NeutrAvidin®).

- 5 Preferably, biotin is conjugated to an alpha chain of a $Fc_\epsilon R$. Preferably a carbohydrate group of the $Fc_\epsilon R$ alpha chain is conjugated to biotin.

In one embodiment, a complex is detected by contacting a putative IgE-containing composition with a canine $Fc_\epsilon R$ molecule that is conjugated to a detectable marker. A suitable detectable marker to conjugate to a $Fc_\epsilon R$ molecule includes, but is
10 not limited to, a radioactive label, a fluorescent label, a chemiluminescent label or a chromophoric label. A detectable marker is conjugated to a $Fc_\epsilon R$ molecule or a reagent in such a manner as not to block the ability of the $Fc_\epsilon R$ or reagent to bind to the IgE being detected. Preferably, a carbohydrate group of a $Fc_\epsilon R$ is conjugated to biotin.

In another embodiment, a $Fc_\epsilon R$ molecule:IgE complex is detected by contacting a
15 putative IgE-containing composition with a $Fc_\epsilon R$ molecule and then contacting the complex with an indicator molecule. Suitable indicator molecules of the present invention include molecules that can bind to either the $Fc_\epsilon R$ molecule or to the IgE antibody. As such, an indicator molecule can comprise, for example, a $Fc_\epsilon R$ molecule, an antigen, an antibody and a lectin, depending upon which portion of the $Fc_\epsilon R$
20 molecule:IgE complex is being detected. Preferred identifying labeled compounds that are antibodies include, for example, anti-IgE antibodies and anti- $Fc_\epsilon R$ antibodies. Preferred lectins include those lectins that bind to high-mannose groups. More preferred lectins bind to high-mannose groups present on a $Fc_\epsilon R$ molecule of the present invention produced in insect cells. An indicator molecule itself can be attached to a detectable
25 marker of the present invention. For example, an antibody can be conjugated to biotin, horseradish peroxidase, alkaline phosphatase or fluorescein.

In one preferred embodiment, a $Fc_\epsilon R$ molecule:IgE complex is detected by contacting the complex with a reagent that selectively binds to a $Fc_\epsilon R$ molecule of the present invention. Examples of such a reagent include, but are not limited to, an
30 antibody that selectively binds to a $Fc_\epsilon R$ molecule (referred to herein as an anti- $Fc_\epsilon R$ antibody) or a compound that selectively binds to a detectable marker conjugated to a

Fc_εR molecule. Fc_εR molecules conjugated to biotin are preferably detected using streptavidin, more preferably using ImmunoPure® NeutrAvidin® (available from Pierce, Rockford, IL).

In another preferred embodiment, a Fc_εR molecule:IgE complex is detected by contacting the complex with a reagent that selectively binds to an IgE antibody (referred to herein as an anti-IgE reagent). Examples of such an anti-IgE reagent include, but are not limited to, a secondary antibody that is an anti-isotype antibody (e.g., an antibody that selectively binds to the constant region of an IgE), an antibody-binding bacterial surface protein (e.g., Protein A or Protein G), an antibody-binding cell (e.g., a B cell, a T cell, a natural killer cell, a polymorphonuclear leukocyte cell, a monocyte cell or a macrophage cell), an antibody-binding eukaryotic cell surface protein (e.g., an Fc receptor), and an antibody-binding complement protein. Preferred anti-IgE reagents include, but are not limited to, D9 (provided by Doug DeBoer, University of Wisconsin), and CMI antibody #9, CMI antibody #19, CMI antibody #59 and CMI antibody #71 (available from Custom Monoclonal International, West Sacramento, CA). In particular, as used herein, an anti-IgE antibody includes not only a complete antibody but also any subunit or portion thereof that is capable of selectively binding to an IgE heavy chain constant region. For example, a portion of an anti-IgE reagent can include an Fab fragment or a F(ab')₂ fragment, which are described in detail in Janeway et al., in *Immunobiology, the Immune System in Health and Disease*, Garland Publishing, Inc., NY, 1996 (which is incorporated herein by this reference in its entirety).

In one embodiment a complex can be formed and detected in solution. In another embodiment, a complex can be formed in which one or more members of the complex are immobilized on (e.g., coated onto) a substrate. Immobilization techniques are known to those skilled in the art. Suitable substrate materials include, but are not limited to, plastic, glass, gel, celluloid, paper, PVDF (poly-vinylidene-fluoride), nylon, nitrocellulose, and particulate materials such as latex, polystyrene, nylon, nitrocellulose, agarose and magnetic resin. Suitable shapes for substrate material include, but are not limited to, a well (e.g., microtiter dish well), a plate, a dipstick, a bead, a lateral flow apparatus, a membrane, a filter, a tube, a dish, a celluloid-type matrix, a magnetic particle, and other particulates. A particularly preferred substrate comprises an ELISA

plate, a dipstick, a radioimmunoassay plate, agarose beads, plastic beads, latex beads, immunoblot membranes and immunoblot papers. In one embodiment, a substrate, such as a particulate, can include a detectable marker.

A preferred method to detect canine IgE is an immunosorbent assay. An
5 immunoabsorbent assay of the present invention comprises a capture molecule and an indicator molecule. A capture molecule of the present invention binds to an IgE in such a manner that the IgE is immobilized to a substrate. As such, a capture molecule is preferably immobilized to a substrate of the present invention prior to exposure of the capture molecule to a putative IgE-containing composition. An indicator molecule of
10 the present invention detects the presence of an IgE bound to a capture molecule. As such, an indicator molecule preferably is not immobilized to the same substrate as a capture molecule prior to exposure of the capture molecule to a putative IgE-containing composition.

A preferred immunoabsorbent assay method includes a step of either: (a) binding
15 a canine $Fc_\epsilon R$ molecule to a substrate prior to contacting a canine $Fc_\epsilon R$ molecule with a putative IgE-containing composition to form a canine $Fc_\epsilon R$ molecule-coated substrate; or (b) binding a putative canine IgE-containing composition to a substrate prior to contacting a canine $Fc_\epsilon R$ molecule with a putative IgE-containing composition to form a putative IgE-containing composition-coated substrate. Preferably, the substrate is a non-
20 coated substrate, an antigen-coated substrate or an anti-IgE antibody-coated substrate.

Both a capture molecule and an indicator molecule of the present invention are capable of binding to an IgE. Preferably, a capture molecule binds to a different region of an IgE than an indicator molecule, thereby allowing a capture molecule to be bound to an IgE at the same time as an indicator molecule. The use of a reagent as a capture
25 molecule or an indicator molecule depends upon whether the molecule is immobilized to a substrate when the molecule is exposed to an IgE. For example, a canine $Fc_\epsilon R$ molecule of the present invention is used as a capture molecule when the $Fc_\epsilon R$ molecule is bound to a substrate. Alternatively, a canine $Fc_\epsilon R$ molecule is used as an indicator molecule when the $Fc_\epsilon R$ molecule is not bound to a substrate. Suitable molecules for
30 use as capture molecules or indicator molecules include, but are not limited to, a canine

Fc_εR molecule of the present invention, an antigen reagent or an anti-IgE antibody reagent of the present invention.

An immunoabsorbent assay of the present invention can further comprise one or more layers and/or types of secondary molecules or other binding molecules capable of
5 detecting the presence of an indicator molecule. For example, an untagged (i.e., not conjugated to a detectable marker) secondary antibody that selectively binds to an indicator molecule can be bound to a tagged (i.e., conjugated to a detectable marker) tertiary antibody that selectively binds to the secondary antibody. Suitable secondary antibodies, tertiary antibodies and other secondary or tertiary molecules can be selected
10 by those of skill in the art. Preferred secondary molecules of the present invention include an antigen, an anti-IgE idiotypic antibody and an anti-IgE isotypic antibody. Preferred tertiary molecules can be selected by a skilled artisan based upon the characteristics of the secondary molecule. The same strategy is applied for subsequent layers.

15 In one embodiment, a desired antigen is used as a capture molecule by being immobilized on a substrate, such as a microtiter dish well or a dipstick. Preferred antigens include those disclosed herein. A biological sample collected from an animal is applied to the substrate and incubated under conditions suitable (i.e., sufficient) to allow for antigen:IgE complex formation bound to the substrate (i.e., IgE in a sample binds to
20 an antigen immobilized on a substrate). Excess non-bound material (i.e., material from the biological sample that has not bound to the antigen), if any, is removed from the substrate under conditions that retain antigen:IgE complex binding to the substrate. Preferred conditions are described generally in Sambrook et al., *ibid.* An indicator molecule that can selectively bind to an IgE bound to the antigen is added to the
25 substrate and incubated to allow formation of a complex between the indicator molecule and the antigen:IgE complex. The indicator molecule can be conjugated to a detectable marker (preferably to an enzyme label, to a colorimetric label, to a fluorescent label, to a radioisotope, or to a ligand such as of the biotin or avidin family). Excess indicator molecule is removed, a developing agent is added if required, and the substrate is
30 submitted to a detection device for analysis. A preferred indicator molecule for this

embodiment is a canine $Fc_\epsilon R$ molecule, preferably conjugated to biotin, to a fluorescent label or to an enzyme label.

In one embodiment, a canine $Fc_\epsilon R$ molecule is used as a capture molecule by being immobilized on a substrate, such as a microtiter dish well or a dipstick. A
5 biological sample collected from an animal is applied to the substrate and incubated under conditions suitable to allow for $Fc_\epsilon R$ molecule:IgE complex formation bound to the substrate. Excess non-bound material, if any, is removed from the substrate under conditions that retain $Fc_\epsilon R$ molecule:IgE complex binding to the substrate. An indicator molecule that can selectively bind to an IgE bound to the $Fc_\epsilon R$ is added to the substrate
10 and incubated to allow formation of a complex between the indicator molecule and the $Fc_\epsilon R$ molecule:IgE complex. Preferably, the indicator molecule is conjugated to a detectable marker, preferably to an enzyme label, to a colorimetric label, to a fluorescent label, to a radioisotope, or to a ligand such as of the biotin or avidin family. Excess indicator molecule is removed, a developing agent is added if required, and the substrate
15 is submitted to a detection device for analysis. A preferred indicator molecule for this embodiment is an antigen that will bind to IgE in the biological sample or an anti-IgE isotype or idiotype antibody, either preferably being conjugated to fluorescein, an enzyme or biotin.

In one embodiment, an anti-IgE antibody (c.g., isotype- or idiotype-specific
20 antibody) is used as a capture molecule by being immobilized on a substrate, such as a microtiter dish well or a dipstick. A biological sample collected from a canine is applied to the substrate and incubated under conditions suitable to allow for anti-IgE antibody:IgE complex formation bound to the substrate. Excess non-bound material, if any, is removed from the substrate under conditions that retain anti-IgE antibody:IgE
25 complex binding to the substrate. A canine $Fc_\epsilon R$ molecule is added to the substrate and incubated to allow formation of a complex between the canine $Fc_\epsilon R$ molecule and the anti-IgE antibody:IgE complex. Preferably, the canine $Fc_\epsilon R$ molecule is conjugated to a detectable marker (preferably to biotin, an enzyme label or a fluorescent label). Excess $Fc_\epsilon R$ molecule is removed, a developing agent is added if required, and the substrate is
30 submitted to a detection device for analysis.

In one embodiment, an immunosorbent assay of the present invention does not utilize a capture molecule. In this embodiment, a biological sample collected from a canine is applied to a substrate, such as a microtiter dish well or a dipstick, and incubated under conditions suitable to allow for IgE binding to the substrate. Any IgE
5 present in the bodily fluid is immobilized on the substrate. Excess non-bound material, if any, is removed from the substrate under conditions that retain IgE binding to the substrate. A canine $Fc_\epsilon R$ molecule is added to the substrate and incubated to allow formation of a complex between the canine $Fc_\epsilon R$ molecule and canine IgE. Preferably, the $Fc_\epsilon R$ molecule is conjugated to a detectable marker (preferably to biotin, an enzyme
10 label or a fluorescent label). Excess $Fc_\epsilon R$ molecule is removed, a developing agent is added if required, and the substrate is submitted to a detection device for analysis.

Another preferred method to detect canine IgE is a lateral flow assay, examples of which are disclosed in U.S. Patent No. 5,424,193, issued June 13, 1995, by Pronovost et al.; U.S. Patent No. 5,415,994, issued May 16, 1995, by Imrich et al; WO 94/29696,
15 published December 22, 1994, by Miller et al.; and WO 94/01775, published January 20, 1994, by Pawlak et al.; each of these patent publications is incorporated by reference herein in its entirety. In one embodiment, a biological sample is placed in a lateral flow apparatus that includes the following components: (a) a support structure defining a flow path; (b) a labeling reagent comprising a bead conjugated to an antigen, the labeling
20 reagent being impregnated within the support structure in a labeling zone; and (c) a capture reagent comprising a canine IgE-binding composition. Preferred antigens include those disclosed herein. The capture reagent is located downstream of the labeling reagent within a capture zone fluidly connected to the labeling zone in such a manner that the labeling reagent can flow from the labeling zone into the capture zone.
25 The support structure comprises a material that does not impede the flow of the beads from the labeling zone to the capture zone. Suitable materials for use as a support structure include ionic (i.e., anionic or cationic) material. Examples of such a material include, but are not limited to, nitrocellulose (NC), PVDF and carboxymethylcellulose (CM). The support structure defines a flow path that is lateral and is divided into zones,
30 namely a labeling zone and a capture zone. The apparatus can further comprise a sample receiving zone located along the flow path, more preferably upstream of the labeling

reagent. The flow path in the support structure is created by contacting a portion of the support structure downstream of the capture zone, preferably at the end of the flow path, to an absorbent capable of absorbing excess liquid from the labeling and capture zones.

In this embodiment, the biological sample is applied to the sample receiving zone which includes a portion of the support structure. The labeling zone receives the sample from the sample receiving zone which is directed downstream by the flow path. The labeling zone comprises the labeling reagent that binds to IgE. A preferred labeling reagent is an antigen conjugated, either directly or through a linker, to a plastic bead substrate, such as to a latex bead. The substrate also includes a detectable marker, preferably a colorimetric marker. Typically, the labeling reagent is impregnated to the support structure by drying or lyophilization. The sample structure also comprises a capture zone downstream of the labeling zone. The capture zone receives labeling reagent from the labeling zone which is directed downstream by the flow path. The capture zone contains the capture reagent, preferably a canine $Fc_\epsilon R$ molecule of the present invention that immobilizes canine IgE complexed to the antigen in the capture zone. The capture reagent is preferably fixed to the support structure by drying or lyophilization. The labeling reagent accumulates in the capture zone and the accumulation is assessed visually or by an optical detection device.

In another embodiment, a lateral flow apparatus used to detect canine IgE includes: (a) a support structure defining a flow path; (b) a labeling reagent comprising a canine $Fc_\epsilon R$ molecule of the present invention, the labeling reagent impregnated within the support structure in a labeling zone; and (c) a capture reagent comprising an antigen, the capture reagent being located downstream of the labeling reagent within a capture zone fluidly connected to the labeling zone in such a manner that the labeling reagent can flow from the labeling zone into the capture zone. The apparatus preferably also includes a sample receiving zone located along the flow path, preferably upstream of the labeling reagent. The apparatus preferably also includes an absorbent located at the end of the flow path.

One embodiment of the present invention is an inhibition assay in which the presence of canine IgE in a putative canine IgE-containing composition is determined by adding such composition to a canine $Fc_\epsilon R$ molecule of the present invention and an

-20-

isolated canine IgE known to bind to the $Fc_\epsilon R$ molecule. The absence of binding of the $Fc_\epsilon R$ molecule to the known IgE indicating the presence of IgE in the putative IgE-containing composition.

The present invention also includes kits to detect canine IgE based, for example, on the disclosed detection methods. One embodiment is a kit to detect canine IgE comprising a canine Fc_ϵ receptor ($Fc_\epsilon R$) molecule and a means for detecting a canine IgE. Suitable and preferred canine $Fc_\epsilon R$ molecules are disclosed herein. Suitable means of detection include compounds disclosed herein that bind to either the canine $Fc_\epsilon R$ molecule or to a canine IgE. A preferred kit of the present invention further comprises a detection means including one or more antigens such as those disclosed herein, an antibody capable of selectively binding to canine IgE such as those disclosed herein and/or a compound capable of binding to a detectable marker conjugated to a canine $Fc_\epsilon R$ molecule (e.g., avidin, streptavidin and ImmunoPurc® NeutrAvidin when the detectable marker is biotin).

A preferred embodiment of a kit of the present invention is a flea allergen kit comprising a flea allergen such as those disclosed herein. A particularly preferred flea allergen for use with a flea allergen kit includes a flea saliva product or a flea saliva protein.

Another preferred kit of the present invention is a general allergen kit comprising an allergen common to all regions of the United States and a canine $Fc_\epsilon R$ molecule of the present invention. As used herein, a "general allergen" kit refers to a kit comprising allergens that are found substantially throughout the United States (i.e., essentially not limited to certain regions of the United States). A general allergen kit provides an advantage over regional allergen kits because a single kit can be used to test a canid from any geographical location in the United States. Suitable and preferred general allergens for use with a general allergen kit of the present invention include those general allergens disclosed herein.

Another preferred kit of the present invention is a food allergen kit comprising (a) a food allergen such as beef, chicken, pork, a mixture of fish, such as cod, halibut or and tuna, egg, milk, Brewer's yeast, whole wheat, corn, soybean, cheese and/or rice, and

(b) a canine FcεR molecule of the present invention. Preferably, the beef, chicken, pork, fish, corn and rice, are cooked.

A preferred kit of the present invention is one in which the allergen is immobilized to a substrate. If a kit comprises two or more antigens, the kit can comprise one or more compositions, each composition comprising one antigen. As such, each antigen can be tested separately. A kit can also contain two or more diagnostic reagents for detecting canine IgE, additional isolated canine IgE antigens and/or antibodies as disclosed herein. Particularly preferred are kits used in a lateral flow assay format. It is within the scope of the present invention that a lateral flow assay kit can include one or more lateral flow assay apparatuses. Multiple lateral flow apparatuses can be attached to each other at one end of each apparatus, thereby creating a fan-like structure.

In particular, a method and kit of the present invention are useful for diagnosing abnormal conditions in animals that are associated with changing levels of canine IgE. Particularly preferred conditions to diagnose include allergies, parasitic infections and neoplasia. For example, a method and kit of the present invention are particularly useful for detecting flea allergy dermatitis (FAD), when such method or kit includes the use of a flea saliva antigen. FAD is defined as a hypersensitive response to fleabites. Preferably, a putative IgE-containing composition is obtained from an animal suspected of having FAD. In addition, methods and kits of the present invention are particularly useful for detecting helminth infection, in particular heartworm infection, when such methods or kits include the use of a helminth antigen, such as Di33. Preferably, a putative canine IgE-containing composition is obtained from a canine suspected of having a helminth infection.

The following examples are provided for the purposes of illustration and are not intended to limit the scope of the present invention.

EXAMPLES

It is to be noted that the Examples include a number of molecular biology, microbiology, immunology and biochemistry techniques considered to be known to those skilled in the art. Disclosure of such techniques can be found, for example, in Sambrook et al., *ibid.*, and related references.

Example 1

This example describes the construction of recombinant baculoviruses expressing a truncated portion of the α chain of canine Fc_ϵ receptor.

Recombinant molecules pVL-ncFc $_\epsilon$ R α 1₆₀₉, pVL-ncFc $_\epsilon$ R α 2₆₀₉, and pVL-ncFc $_\epsilon$ R α 3₆₁₇, each containing nucleic acid molecules encoding the extracellular domain of the canine Fc $_\epsilon$ R α chain, operatively linked to baculovirus polyhedron transcription control sequences were produced in the following manner. Three different canine Fc $_\epsilon$ R α chain extracellular domain nucleic acid molecule-containing fragments, each of about 608 to about 609 nucleotides were amplified by polymerase chain reaction (PCR) from either a canine splenic mononuclear cell cDNA library or a canine lymph node mononuclear cell cDNA library, each library produced using standard techniques, using a forward primer CIERMet containing a *Bam*HI site, having the nucleic acid sequence 5'-TGC GGA TCC AAT ATG CCT GCT TCC ATG GGA G-3' (denoted SEQ ID NO:1) and a reverse primer CIERSec containing an *Eco*RI site, having the nucleic acid sequence 5'-TTG GAA TTC TTA CTC TTT TTT CAC AAT AAT GTT G-3' (denoted herein as SEQ ID NO:2). The resulting PCR products were digested with *Bam*HI and *Eco*RI to produce the following nucleic acid molecules: ncFc $_\epsilon$ R α 1₆₀₉ (also denoted ncFc $_\epsilon$ R α LN4₆₀₉), ncFc $_\epsilon$ R α 2₆₀₉ (also denoted ncFc $_\epsilon$ R α SPL6₆₀₉) and ncFc $_\epsilon$ R α 3₆₁₇ (also denoted ncFc $_\epsilon$ R α SPL3R₆₁₇). Nucleic acid molecule ncFc $_\epsilon$ R α 1₆₀₉ was obtained from the PCR reaction derived from the canine lymph node mononuclear cell cDNA library. Nucleic acid molecules ncFc $_\epsilon$ R α 2₆₀₉ and ncFc $_\epsilon$ R α 3₆₁₇ were obtained from the PCR reaction derived from the canine splenic mononuclear cell cDNA library. Nucleic acid molecules ncFc $_\epsilon$ R α 1₆₀₉, ncFc $_\epsilon$ R α 2₆₀₉, and ncFc $_\epsilon$ R α 3₆₁₇ each were sequenced by the Sanger dideoxy chain termination method, using the PRISMTM Ready Dye Terminator Cycle Sequencing Kit with Ampli Taq DNA Polymerase, FS (available from the Perkin-Elmer Corporation, Norwalk, CT). Nucleic acid molecules ncFc $_\epsilon$ R α 1₆₀₉, ncFc $_\epsilon$ R α 2₆₀₉, and ncFc $_\epsilon$ R α 3₆₁₇ each contained an about 608 to an about 609 nucleotide fragment encoding the extracellular domain of the canine Fc $_\epsilon$ R α chain, the coding strands of which have nucleic acid sequences denoted SEQ ID NO:3, SEQ ID NO:8, and SEQ ID NO:13, respectively. The complement of SEQ ID NO:3 is represented herein by SEQ

ID NO:5. The complement of SEQ ID NO:8 is represented herein by SEQ ID NO:10.
The complement of SEQ ID NO:13 is represented herein by SEQ ID NO:15.

Translation of SEQ ID NO:3 indicates that nucleic acid molecule ncFc_εRα1₆₀₉ encodes a Fc_εR protein of about 197 amino acids, referred to herein as PcFc_εRα1₁₉₇,
5 having amino acid sequence SEQ ID NO:4, assuming an open reading frame having a start codon spanning from about nucleotide 10 through about nucleotide 12 of SEQ ID NO:3 and a stop codon spanning from about nucleotide 601 through about nucleotide 603 of SEQ ID NO:3. This open reading frame, excluding the stop codon, comprises nucleic acid molecule ncFc_εRα1₅₉₁ of the present invention, the nucleic acid sequence of
10 which is represented herein by SEQ ID NO:6. The complement of SEQ ID NO:6 is represented herein by SEQ ID NO:7.

Translation of SEQ ID NO:8 indicates that nucleic acid molecule ncFc_εRα2₆₀₉ encodes a Fc_εR protein of about 197 amino acids, referred to herein as PcFc_εRα2₁₉₇,
having amino acid sequence SEQ ID NO:9, assuming an open reading frame having a
15 start codon spanning from about nucleotide 10 through about nucleotide 12 of SEQ ID NO:8 and a stop codon spanning from about nucleotide 601 through about nucleotide 603 of SEQ ID NO:8. This open reading frame, excluding the stop codon, comprises nucleic acid molecule ncFc_εRα2₅₉₁ of the present invention, the nucleic acid sequence of which is represented herein by SEQ ID NO:11. The complement of SEQ ID NO:11 is
20 represented herein by SEQ ID NO:12.

Translation of SEQ ID NO:13 indicates that nucleic acid molecule ncFc_εRα3₆₁₇ encodes a Fc_εR protein of about 199 amino acids, referred to herein as PcFc_εRα3₁₉₉,
having amino acid sequence SEQ ID NO:14, assuming that the initiation codon spans from about nucleotide 10 through about nucleotide 12 of SEQ ID NO:13 and the last
25 codon spans from about nucleotide 595 through about nucleotide 597 of SEQ ID NO:13. This open reading frame comprises nucleic acid molecule ncFc_εRα3₅₉₇ of the present invention, the nucleic acid sequence of which is represented herein by SEQ ID NO:16. The complement of SEQ ID NO:16 is represented herein by SEQ ID NO:17.

In order to produce baculovirus recombinant molecules capable of directing the
30 production of PcFc_εRα1₁₉₇, PcFc_εRα2₁₉₇, and PcFc_εRα3₁₉₉, nucleic acid molecules ncFc_εRα1₆₀₉, ncFc_εRα2₆₀₉, and ncFc_εRα3₆₁₇ were subcloned into unique *Bam*HI and

*Eco*RI sites of pVL1393 baculovirus shuttle plasmid (available from Pharmingen, San Diego, CA) to produce recombinant molecules referred to herein as pVL-ncFc_εRα1₆₀₉, pVL-ncFc_εRα2₆₀₉, and pVL-ncFc_εRα3₆₁₇, respectively. The resultant recombinant molecules pVL-ncFc_εRα1₆₀₉, pVL-ncFc_εRα2₆₀₉, and pVL-ncFc_εRα3₆₁₇ were verified for proper insert orientation by restriction mapping.

Recombinant molecules pVL-ncFc_εRα1₆₀₉, pVL-ncFc_εRα2₆₀₉, and pVL-ncFc_εRα3₆₁₇ were co-transfected with a linear Baculogold™ baculovirus DNA (available from Pharmingen) into *S. frugiperda* Sf9 cells (available from Invitrogen Corp., San Diego, CA) using methods prescribed by the manufacturer to form recombinant cells *S. frugiperda*:pVL-ncFc_εRα1₆₀₉, *S. frugiperda*:pVL-ncFc_εRα2₆₀₉, and *S. frugiperda*:pVL-ncFc_εRα3₆₁₇. Recombinant baculoviruses were plaque purified and amplified from each transfection by methods well known to those skilled in the art, to produce recombinant baculoviruses BV-ncFc_εRα1₆₀₉, BV-ncFc_εRα2₆₀₉, and BV-ncFc_εRα3₆₁₇, respectively.

Example 2

This example describes the production of PcFc_εRα1₁₉₇, PcFc_εRα2₁₉₇, and PcFc_εRα3₁₉₉ canine Fc_εR α chain proteins.

About 1.5 liter cultures of serum-free ex-Cell Medium (available from Invitrogen) were seeded with about 1×10^6 *Trichoplusia ni* cells (High Five™ cells; available from Invitrogen) per milliliters (ml) of medium. The cell cultures were inoculated with recombinant baculoviruses BV-ncFc_εRα1₆₀₉, BV-ncFc_εRα2₆₀₉, and BV-ncFc_εRα3₆₁₇, respectively, at multiplicities of infection (MOI) of about 2 to about 5 plaque forming units (pfu) per cell to produce recombinant cells *Trichoplusia ni*-BV-ncFc_εRα1₆₀₉, *Trichoplusia ni*-BV-ncFc_εRα2₆₀₉, and *Trichoplusia ni*-BV-ncFc_εRα3₆₁₇. The infections were allowed to proceed at a controlled temperature of 27°C for 48 hours, to produce recombinant proteins of PcFc_εRα1₁₉₇, PcFc_εRα2₁₉₇, and PcFc_εRα3₁₉₉. Following infection, cells were separated from the medium by centrifugation, and the medium was frozen at -70°C.

Example 3

This example describes the binding of PcFc_εRα1₁₉₇, PcFc_εRα2₁₉₇, or PcFc_εRα3₁₉₉ protein to canine IgE.

About 4.5 ml of the culture media described immediately above containing PcFc_εRα1₁₉₇, PcFc_εRα2₁₉₇, or PcFc_εRα3₁₉₉, respectively, were loaded onto columns comprising a canine IgE monoclonal antibody (a gift from Chris Grant, Custom Monoclonals International, West Sacramento, CA) linked to sepharose 4B. Each column was washed with about 4 ml of carbonate buffer (0.1 M NaHCO₃, pH 8.3 and 0.5 M NaCl). Protein bound to the IgE on each column was eluted from the column using about 3 ml of 0.1 M glycine-HCl, pH 2.8. Each column was further washed with about 1 ml of carbonate buffer and then with about 4 ml of buffer comprising 0.1 M NaHCO₃, pH 8.3. The elution samples and wash samples from a given column were combined and concentrated to a volume of about 0.35 ml. The eluted protein from each column was resolved on separate 14% Tris-glycine polyacrylamide-SDS gels. The gels were then stained with coomassie stain. A diffused band was observed at about 31 kilodaltons (kD).

Amino (N-) terminal amino acid sequencing analysis was performed on protein contained in the diffused band using standard procedures known to those in the art (see, for example, Geisow et al., 1989, in *Protein Sequencing: A Practical Approach*, JBC Findlay and MJ Geisow (eds.), IRL Press, Oxford, England, pp. 85-98; Hewick et al., 1981, *J. Biol. Chem.*, Vol. 256, pp. 7990-7997). The N-terminal partial amino acid sequence of a protein contained in the band was determined to be S D T L K P T V X M N P P X N L I (as represented in standard single letter code, and denoted herein as SEQ ID NO:18; "X" represents any amino acid). Comparison of SEQ ID NO:18 and the amino acid sequence of the canine Fc_εR alpha chain reported in Hayashi et al., *ibid.*, indicated that PcFc_εRα1₁₉₇, PcFc_εRα2₁₉₇, and PcFc_εRα3₁₉₉, expressed in baculovirus, each bound to canine IgE antibodies.

25 Example 4

This example describes the isolation, by DNA hybridization, and sequencing of a nucleic acid molecule encoding the Fc_εR α chain from *Canis canis*.

A. Isolation of nucleic acid molecule ncFc_εRα4₁₉₉₁

A nucleic acid molecule was isolated from a canine mast cell cDNA library by the molecule's ability to hybridize with a ³²P-labeled probe derived from a PCR clone encoding the canine Fc_εR α chain. The canine mast cell cDNA library was prepared

using standard techniques. Using a modification of the protocol described in the cDNA Synthesis Kit, the mast cell cDNA library was screened, using duplicate plaque lifts, with a ^{32}P -labeled probe comprising ncFc $_{\epsilon}$ R α 1 $_{609}$ (SEQ ID NO:3). A plaque purified clone containing a canine nucleic acid molecule encoding the Fc $_{\epsilon}$ R α chain was
5 converted into a double stranded recombinant molecule, using the ExAssistTM helper phage and SOLRTM *E. coli* according to the *in vivo* excision protocol described in the ZAP-cDNA Synthesis Kit (available from Stratagene). Double-stranded plasmid DNA was prepared using an alkaline lysis protocol, such as that described in Sambrook et al., *ibid.* The plasmid comprised a canine Fc $_{\epsilon}$ R α chain nucleic acid molecule of about 991
10 nucleotides denoted herein as ncFc $_{\epsilon}$ R α 4 $_{991}$.

B. Sequence analysis of nucleic acid molecule ncFc $_{\epsilon}$ R α 4 $_{991}$

The nucleic acid molecule ncFc $_{\epsilon}$ R α 4 $_{991}$ was sequenced by standard Sanger dideoxy chain termination sequencing techniques (see, for example, Sambrook et al., *ibid.*). DNA sequence analysis, including the compilation of sequences and the
15 determination of open reading frames, were performed using the MacVectorTM program (available from the Eastman Kodak Company, New Haven, CT), or the DNAsisTM program (available from Hitachi Software, San Bruno, CA). Protein sequence analysis, including the determination of molecular weight and isoelectric point (pI) was performed using the MacVectorTM program.

20 The nucleic acid sequence of the coding strand of ncFc $_{\epsilon}$ R α 4 $_{991}$ is denoted herein as SEQ ID NO:19. Translation of SEQ ID NO:19 suggests that nucleic acid molecule ncFc $_{\epsilon}$ R α 4 $_{991}$ encodes a full-length canine Fc $_{\epsilon}$ R α chain protein of about 253 amino acids, referred to herein as PcFc $_{\epsilon}$ R α 4 $_{253}$, having amino acid sequence SEQ ID NO:20, assuming an open reading frame in which the initiation codon spans from about
25 nucleotide 35 through about nucleotide 37 of SEQ ID NO:19 and the termination codon spans from about nucleotide 794 through about nucleotide 796 of SEQ ID NO:19. The complement of SEQ ID NO:20 is represented herein by SEQ ID NO:21. The coding region encoding PcFc $_{\epsilon}$ R α 4 $_{253}$, is represented by nucleic acid molecule ncFc $_{\epsilon}$ R α 4 $_{759}$, having a coding strand with the nucleic acid sequence represented by SEQ ID NO:22
30 and a complementary strand with nucleic acid sequence SEQ ID NO:23. The amino acid

sequence of PcFc_εRα₂₅₃ (i.e., SEQ ID NO:21) predicts that PcFc_εRα₂₅₃ has an estimated molecular weight of about 28.5 kD and an estimated pI of about 9.62.

Analysis of SEQ ID NO: 20 suggests the presence of a signal peptide encoded by a stretch of amino acids spanning from about amino acid 1 through about amino acid 24. The proposed mature protein, denoted herein as PcFc_εRα₄₂₂₉, contains about 229 amino acids, the sequence of which is shown as SEQ ID NO:24. The coding strand encoding PcFc_εRα₄₂₂₉ is represented herein as SEQ ID NO:30. The amino acid sequence of PcFc_εRα₄₂₂₉ (i.e., SEQ ID NO:24) predicts that PcFc_εRα₄₂₂₉ has an estimated molecular weight of about 26 kD, an estimated pI of about 9.65 and five predicted asparagine-linked glycosylation sites extending from about amino acids 29-31, 42-44, 71-73, 135-137 and 148-150, respectively.

Comparison of amino acid sequence SEQ ID NO:20 with amino acid sequences reported in GenBank indicates that SEQ ID NO:20 showed the most homology, i.e., about 100% identity between SEQ ID NO:20 and a *Canis canis* Fc_εR α chain protein (GenBank accession number D16413). Comparison of amino acid sequence SEQ ID NO:22 with nucleic acid sequences reported in GenBank indicates that SEQ ID NO:22 showed the most homology, i.e., about 100% identity between SEQ ID NO:22 and a canine mRNA for Fc_εR α chain (GenBank accession D16413).

Example 5

This Example demonstrates the production of secreted canine Fc_εR α chain protein in eukaryotic cells.

To produce a secreted form of a canine Fc_εR α chain, recombinant molecule pVL-ncFc_εRα₄₅₉₁, containing a canine Fc_εR α chain nucleic acid molecule encoding a secreted form of canine Fc_εR α chain spanning nucleotides from about 35 through about 625 of SEQ ID NO:19 operatively linked to baculovirus polyhedron transcription control sequences, was produced in the following manner. A canine Fc_εR α chain nucleic acid molecule of about 591 nucleotides was PCR amplified from ncFc_εRα₄₉₉₁ DNA using a sense primer canIgEr FWD having the nucleic acid sequence 5' GCG AAG ATC TAT AAA TAT GCC TGC TTC CAT GGG- 3' (SEQ ID NO:25; *Bgl*III site shown in bold) and an antisense primer canIgEr REV having the nucleic acid sequence 5' GCA GGA ATT CTT ACT CTT TTT TCA CAA TAA TGT -3' (SEQ ID NO:26; *Eco*RI site shown

in bold). The N-terminal primer was designed from the pol h sequence of baculovirus with modifications to enhance expression in the baculovirus system.

The about 591 base pair PCR product (referred to as ncFc_cRα_{4,591}) has a coding strand nucleic acid sequence denoted herein as SEQ ID NO:27. The complement of
5 SEQ ID NO:27 is represented herein by SEQ ID NO:29. Translation of SEQ ID NO:27 indicates that nucleic acid molecule ncFc_cRα_{4,591} encodes a Fc_cR α chain protein of about 197 amino acids, referred to herein as PcFc_cRα_{4,197}, having amino acid sequence SEQ ID NO:28. Nucleic acid molecule ncFc_cRα_{4,591} encodes a secretable form of the canine Fc_cR α chain. The processed protein product encoded by ncFc_cRα_{4,591} does not
10 possess a leader sequence or transmembrane domain, and is referred to herein as PcFc_cRα_{4,173}, represented herein by SEQ ID NO:31.

Nucleic acid molecule Bv-ncFc_cRα_{4,591} was digested with *Bgl*III and *Eco*RI and subcloned into the unique *Bgl*III and *Eco*RI sites of baculovirus shuttle plasmid pVL1392 (available from Pharmingen, San Diego, CA) to produce the recombinant molecule
15 referred to herein as pVL-ncFc_cRα_{4,591}. The resultant recombinant molecule, pVL-ncFc_cRα_{4,591}, was verified for proper insert orientation by restriction mapping. The recombinant molecule pVL-ncFc_cRα_{4,591} was co-transfected with a Baculogold™ baculovirus DNA into *S. frugiperda* Sf9 cells (available from Invitrogen) to form recombinant cells denoted *S. frugiperda*:pVL-ncFc_cRα_{4,591}. Recombinant baculovirus
20 was plaque purified and amplified from each transfection by methods well known to those skilled in the art, to produce recombinant baculovirus BV-ncFc_cRα_{4,591}.

S. frugiperda:pVL-ncFc_cRα_{4,591} cells were cultured in order to produce a secreted canine Fc_cR α chain protein, PcFc_cRα_{4,197} in the following manner. An about 1.5 liter cultures of serum-free ex-Cell Medium was seeded with about 1 x 10⁶ *Trichoplusia ni*
25 cells (High Five™ cells) per ml of medium. The cell culture was inoculated with recombinant baculovirus BV-ncFc_cRα_{4,591} at a multiplicity of infection (MOI) of about 2 to about 5 plaque forming units (pfu) per cell to produce recombinant cell *Trichoplusia ni*:BV-ncFc_cRα_{4,591}. The infection was allowed to proceed at a controlled temperature of 27°C for 48 hours, to produce recombinant protein of PcFc_cRα_{4,197}. Following infection,
30 cells were separated from the medium by centrifugation, and the medium was frozen at -70°C.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT:
5 (A) NAME: Heska Corporation
(B) STREET: 1825 Sharp Point Drive
(C) CITY: Fort Collins
(D) STATE: CO
(E) COUNTRY: US
10 (F) POSTAL CODE (ZIP): 80525
(G) TELEPHONE: (970) 493-7272
(H) TELEFAX: (970) 484-9505
- (ii) TITLE OF INVENTION: METHOD TO DETECT CANINE IgE
- (iii) NUMBER OF SEQUENCES: 31
- (iv) CORRESPONDENCE ADDRESS:
15 (A) ADDRESSEE: LAHIVE & COCKFIELD, LLP
(B) STREET: 28 STATE STREET
(C) CITY: BOSTON
(D) STATE: MA
20 (E) COUNTRY: US
(F) ZIP: 02109
- (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
25 (C) OPERATING SYSTEM: Windows 95
(D) SOFTWARE: ASCII DOS TEXT
- (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
30 (A) APPLICATION NUMBER: 08/833,488
(B) FILING DATE: 07-APR-1997
- (viii) ATTORNEY/AGENT INFORMATION:
35 (A) NAME: Rothenberger, Scott D.
(B) REGISTRATION NUMBER: 41,277
(C) REFERENCE/DOCKET NUMBER:
- (ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (617) 227-7400
(B) TELEFAX: (617) 742-4214

40 (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
45 (A) LENGTH: 30 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: primer
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TGCGGATCCA ATATGCCTGC TTCCATGGGA

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 34 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TTGGAATTCT TACTCTTTT TCACAATAAT GTTG

34

10 (2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 609 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURES:
 (A) NAME/KEY: CDS
 (B) LOCATION: 10..603

20 (ix) FEATURES:
 (A) NAME/KEY: R = A or G
 (B) LOCATION: 187

25 (ix) FEATURES:
 (A) NAME/KEY: Xaa = unknown amino acid
 (B) LOCATION: 60

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGATCCAAT ATG CCT GCT TCC ATG GGA GGC CCT GCC CTG CTG 42
 Met Pro Ala Ser Met Gly Gly Pro Ala Leu Leu
 1 5 10

30 TGG CTA GCG CTG CTG CTC TCC TCT CCA GGT GTC ATG TCA TCA 84
 Trp Leu Ala Leu Leu Leu Ser Ser Pro Gly Val Met Ser Ser
 15 20 25

GAT ACC TTG AAA CCT ACA GTG TCC ATG AAC CCG CCA TGG AAT 126
 Asp Thr Leu Lys Pro Thr Val Ser Met Asn Pro Pro Trp Asn
 30 35

ACA ATA TTG AAG GAT GAC AGT GTG ACT CTT ACA TGT ACT GGG 168
 Thr Ile Leu Lys Asp Asp Ser Val Thr Leu Thr Cys Thr Gly
 40 45 50

40 TAC AAC TCC CTT GAA GTC GRC TCT GCT GTG TGG CTC CAC AAC 210
 Asn Asn Ser Leu Glu Val Xaa Ser Ala Val Trp Leu His Asn
 55 60 65

AAC ACT ACT TTG CAA GAG ACG ACT TCA CGT TTG GAC ATC AAT 252
 Asn Thr Thr Leu Gln Glu Thr Thr Ser Arg Leu Asp Ile Asn
 70 75 80

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	AAA GCC CAA ATC CAG GAC AGT GGG GAG TAC AGG TGT CGG GAA	294
	Lys Ala Gln Ile Gln Asp Ser Gly Glu Tyr Arg Cys Arg Glu	
	85 90 95	
5	AAT AGA TCC ATC CTG AGT GAT CCT GTG TAC CTA ACA GTC TTC	336
	Asn Arg Ser Ile Leu Ser Asp Pro Val Tyr Leu Thr Val Phe	
	100 105	
	ACA GAG TGG CTG ATC CTT CAA GCC TCT GCC AAC GTG GTG ATG	378
	Thr Glu Trp Leu Ile Leu Gln Ala Ser Ala Asn Val Val Met	
	110 115 120	
10	GAG GGT GAG AGC TTC CTC ATC AGG TGC CAT AGT TGG AAG AAT	420
	Glu Gly Glu Ser Phe Leu Ile Arg Cys His Ser Trp Lys Asn	
	125 130 135	
15	TTG AGG CTC ACA AAG GTG ACC TAC TAC AAG GAT GGC ATC CCC	462
	Leu Arg Leu Thr Lys Val Thr Tyr Tyr Lys Asp Gly Ile Pro	
	140 145 150	
	ATC AGG TAC TGG TAC GAG AAC TTC AAC ATC TCC ATT AGC AAC	504
	Ile Arg Tyr Trp Tyr Glu Asn Phe Asn Ile Ser Ile Ser Asn	
	155 160 165	
20	GTC ACA ACC AAA AAC AGC GGC AAC TAT TCC TGC TCA GGC CAG	546
	Val Thr Thr Lys Asn Ser Gly Asn Tyr Ser Cys Ser Gly Gln	
	170 175	
	ATC CAG CAG AAA GGC TAC ACC TCT AAA GTC CTC AAC ATT ATT	588
	Ile Gln Gln Lys Gly Tyr Thr Ser Lys Val Leu Asn Ile Ile	
	180 185 190	
25	GTG AAA AAA GAG TAA GAATTC	609
	Val Lys Lys Glu	
	195	

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 197 amino acids
- (B) TYPE: nucleic acid
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (ix) FEATURES:
- (A) NAME/KEY: Xaa = unknown amino acid
- (B) LOCATION: 60
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Pro Ala Ser Met Gly Gly Pro Ala Leu Leu Trp Leu Ala
1 5 10

Leu Leu Leu Ser Ser Pro Gly Val Met Ser Ser Asp Thr Leu
15 20 25

Lys Pro Thr Val Ser Met Asn Pro Pro Trp Asn Thr Ile Leu
30 35 40

Lys Asp Asp Ser Val Thr Leu Thr Cys Thr Gly Asn Asn Ser
45 50 55

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Leu Glu Val Xaa Ser Ala Val Trp Leu His Asn Asn Thr Thr
60 65 70

Leu Gln Glu Thr Thr Ser Arg Leu Asp Ile Asn Lys Ala Gln
75 80

5 Ile Gln Asp Ser Gly Glu Tyr Arg Cys Arg Glu Asn Arg Ser
85 90 95

Ile Leu Ser Asp Pro Val Tyr Leu Thr Val Phe Thr Glu Trp
100 105 110

10 Leu Ile Leu Gln Ala Ser Ala Asn Val Val Met Glu Gly Glu
115 120 125

Ser Phe Leu Ile Arg Cys His Ser Trp Lys Asn Leu Arg Leu
130 135 140

Thr Lys Val Thr Tyr Tyr Lys Asp Gly Ile Pro Ile Arg Tyr
145 150

15 Trp Tyr Glu Asn Phe Asn Ile Ser Ile Ser Asn Val Thr Thr
155 160 165

Lys Asn Ser Gly Asn Tyr Ser Cys Ser Gly Gln Ile Gln Gln
170 175 180

20 Lys Gly Tyr Thr Ser Lys Val Leu Asn Ile Ile Val Lys Lys
185 190 195
Glu

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
- 25 (A) LENGTH: 609 nucleotides
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (ix) FEATURES:
- 30 (A) NAME/KEY: Y = G or T
(B) LOCATION: 422
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

	GAATTCTTAC	TCTTTTTTCA	CAATAATGTT	GAGGACTTTA	GAGGTGTAGC	50
	CTTTCTGCTG	GATCTGGCCT	GAGCAGGAAT	AGTTGCCGCT	GTTTTTGGTT	100
35	GTGACGTTGC	TAATGGAGAT	GTTGAAGTTC	TCGTACCACT	ACCTGATGGG	150
	GATGCCATCC	TTGTAGTAGG	TCACCTTTGT	GAGCCTCAAA	TTCTTCCAAC	200
	TATGGCACCT	GATGAGGAAG	CTCTACCCCT	CCATCACCAC	GTGGGCAGAG	250
	GCTTGAAGGA	TCAGCCACTC	TGTGAAGACT	GTTAGGTACA	CAGGATCACT	300
	CAGGATGGAT	CTATTTTCCC	GACACCTGTA	CTCCCCACTG	TCTTGGATTT	350
40	GGGCTTTATT	GATGTCCAAA	CGTGAAGTCG	TCTCTTGCL	AGTAGTGTTC	400
	TTGTGGAGCC	ACACAGCAGA	GYCGACTTCA	AGGGAGTTGT	TCCCAGTACA	450
	TGTAAGAGTC	ACACTGTCAT	CCTTCAATAT	TGTATTCCAT	GGCGGGTTCA	500
	TGGACACTGT	AGGTTTCAAG	GTATCTGATG	ACATGACACC	TGGAGAGGAG	550
	AGCAGCAGCG	CTAGCCACAG	CAGGGCAGGG	CCTCCCATGG	AAGCAGGCAT	600
45	ATTGGATCC					609

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 591 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURES:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..591

(ix) FEATURES:
 (A) NAME/KEY: R = A or G
 (B) LOCATION: 179

(ix) FEATURES:
 (A) NAME/KEY: Xaa = unknown amino acid
 (B) LOCATION: 60

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

20	ATG CCT GCT TCC ATG GGA GGC CCT GCC CTG CTG TGG CTA GCG Met Pro Ala Ser Met Gly Gly Pro Ala Leu Leu Trp Leu Ala	42
	1 5 10	
	CTG CTG CTC TCC TCT CCA GGT GTC ATG TCA TCA GAT ACC TTG Leu Leu Leu Ser Ser Pro Gly Val Met Ser Ser Asp Thr Leu	84
	15 20 25	
25	AAA CCT ACA GTG TCC ATG AAC CCG CCA TGG AAT ACA ATA TTG Lys Pro Thr Val Ser Met Asn Pro Pro Trp Asn Thr Ile Leu	126
	30 35 40	
	AAG GAT GAC AGT GTG ACT CTT ACA TGT ACT GGG AAC AAC TCC Lys Asp Asp Ser Val Thr Leu Thr Cys Thr Gly Asn Asn Ser	168
	45 50 55	
30	CTT GAA GTC GRC TCT GCT GTG TGG CTC CAC AAC AAC ACT ACT Leu Glu Val Xaa Ser Ala Val Trp Leu His Asn Asn Thr Thr	210
	60 65 70	
35	TTG CAA GAG ACG ACT TCA CGT TTG GAC ATC AAT AAA GCC CAA Leu Gln Glu Thr Thr Ser Arg Leu Asp Ile Asn Lys Ala Gln	252
	75 80	
	ATC CAG GAC AGT GGG GAG TAC AGG TGT CGG GAA AAT AGA TCC Ile Gln Asp Ser Gly Glu Tyr Arg Cys Arg Glu Asn Arg Ser	294
	85 90 95	
40	ATC CTG AGT GAT CCT GTG TAC CTA ACA GTC TTC ACA GAG TGG Ile Leu Ser Asp Pro Val Tyr Leu Thr Val Phe Thr Glu Trp	336
	100 105 110	
	CTG ATC CTT CAA GCC TCT GCC AAC GTG GTG ATG GAG GGT GAG Leu Ile Leu Gln Ala Ser Ala Asn Val Val Met Glu Gly Glu	378
	115 120 125	
45	AGC TTC CTC ATC AGG TGC CAT AGT TGG AAG AAT TTG AGG CTC Ser Phe Leu Ile Arg Cys His Ser Trp Lys Asn Leu Arg Leu	420
	130 135 140	

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ACA AAG GTG ACC TAC TAC AAG GAT GGC ATC CCC ATC AGG TAC 462
 Thr Lys Val Thr Tyr Tyr Lys Asp Gly Ile Pro Ile Arg Tyr
 145 150

5 TGG TAC GAG AAC TTC AAC ATC TCC ATT AGC AAC GTC ACA ACC 504
 Trp Tyr Glu Asn Phe Asn Ile Ser Ile Ser Asn Val Thr Thr
 155 160 165

AAA AAC AGC GGC AAC TAT TCC TGC TCA GGC CAG ATC CAG CAG 546
 Lys Asn Ser Gly Asn Tyr Ser Cys Ser Gly Gln Ile Gln Gln
 170 175 180

10 AAA GGC TAC ACC TCT AAA GTC CTC AAC ATT ATT GTG AAA AAA 588
 Lys Gly Tyr Thr Ser Lys Val Leu Asn Ile Ile Val Lys Lys
 185 190 195

GAG 591
 Glu

15 (2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 591 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURES:
 (A) NAME/KEY: Y = C or T
 (B) LOCATION: 413

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CTCTTTTTC ACAATAATGT TGAGGACTTT AGAGGTGTAG CCTTTCTGCT 50
 GGATCTGGCC TGAGCAGGAA TAGTTGCCGC TGTTTTGGT TGTGACGTTG 100
 CTAATGGAGA TGTGAAGTT CTCGTACCAG TACCTGATGG GGATGCCATC 150
 CTTGTAGTAG GTCACCTTTG TGAGCCTCAA ATTCTTCCAA CTATGGCACC 200
 30 TGATGAGGAA GCTCTACCC TCCATCACCA CGTTGGCAGA GGCTTGAAGG 250
 ATCAGCCACT CTGTGAAGAC TGTTAGGTAC ACAGGATCAC TCAGGATGGA 300
 TCTATTTTCC CGACACCTGT ACTCCCCACT GTCCTGGATT TGGGCTTTAT 350
 TGATGTCCAA ACGTGAAGTC GTCTCTTGCA AAGTAGTGTT GTTGTGGAGC 400
 CACACAGCAG AGYCGACTTC AAGGGAGTTG TTCCCAGTAC ATGTAAGAGT 450
 35 CACACTGTCA TCCTTCAATA TTGTATTCCA TGGCGGGTTC ATGGACACTG 500
 TAGGTTTCAA GGTATCTGAT GACATGACAC CTGGAGAGGA GAGCAGCAGC 550
 GCTAGCCACA GCAGGGCAGG GCCTCCCATG GAAGCAGGCA T 591

(2) INFORMATION FOR SEQ ID NO:8:

40 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 609 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

45 (ix) FEATURES:
 (A) NAME/KEY: CDS
 (B) LOCATION: 10..604

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- (ix) FEATURES:
 (A) NAME/KEY: Xaa = unknown amino acid
 (B) LOCATION: 60, 195, 196
- 5 (ix) FEATURES:
 (A) NAME/KEY: K = G or T
 (B) LOCATION: 187
- (ix) FEATURES:
 (A) NAME/KEY: N = unknown nucleotide
 (B) LOCATION: 592, 595, 596
- 10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
- | | | |
|----|---|-----|
| | GGATCCAAT ATG CCT GCT TCC ATG GGA GGC CCT GCC CTG CTG | 42 |
| | Met Pro Ala Ser Met Gly Gly Pro Ala Leu Leu | |
| | 1 5 10 | |
| 15 | TGG CTA GCG CTG CTG CTC TCC TCT CCA GGT GTC ATG TCA TCA | 84 |
| | Trp Leu Ala Leu Leu Ser Ser Pro Gly Val Met Ser Ser | |
| | 15 20 25 | |
| | GAT ACC TTG AAA CCT ACA GTG TCC ATG AAC CCG CCA TGG AAT | 126 |
| | Asp Thr Leu Lys Pro Thr Val Ser Met Asn Pro Pro Trp Asn | |
| | 30 35 | |
| 20 | ACA ATA TTG AAG GAT GAC AGT GTG ACT CTT ACA TGT ACT GGG | 168 |
| | Thr Ile Leu Lys Asp Asp Ser Val Thr Leu Thr Cys Thr Gly | |
| | 40 45 50 | |
| 25 | AAC AAC TCC CTT GAA GTC GKC TCT GCT GTG TGG CTC CAC AAC | 210 |
| | Asn Asn Ser Leu Glu Val Xaa Ser Ala Val Trp Leu His Asn | |
| | 55 60 65 | |
| | AAC ACT ACT TTG CAA GAG ACG ACT TCA CGT TTG GAC ATC AAT | 252 |
| | Asn Thr Thr Leu Gln Glu Thr Thr Ser Arg Leu Asp Ile Asn | |
| | 70 75 80 | |
| 30 | AAA GCC CAA ATC CAG GAC AGT GGG GAG TAC AGG TGT CGG GAA | 294 |
| | Lys Ala Gln Ile Gln Asp Ser Gly Glu Tyr Arg Cys Arg Glu | |
| | 85 90 95 | |
| | AAT AGA TCC ATC CTG AGT GAT CCT GTG TAC CTA ACA GTC TTC | 336 |
| | Asn Arg Ser Ile Leu Ser Asp Pro Val Tyr Leu Thr Val Phe | |
| | 100 105 | |
| 35 | ACA GAG TGG CTG ATC CTT CAA GCC TCT GCC AAC GTG GTG ATG | 378 |
| | Thr Glu Trp Leu Ile Leu Gln Ala Ser Ala Asn Val Val Met | |
| | 110 115 120 | |
| 40 | GAG GGT GAG AGC TTC CTC ATC AGG TGC CAT AGT TGG AAG AAT | 420 |
| | Glu Gly Glu Ser Phe Leu Ile Arg Cys His Ser Trp Lys Asn | |
| | 125 130 135 | |
| | TTG AGG CTC ACA AAG GTG ACC TAC TAC AAG GAT GGC ATC CCC | 452 |
| | Leu Arg Leu Thr Lys Val Thr Tyr Tyr Lys Asp Gly Ile Pro | |
| | 140 145 150 | |
| 45 | ATC AGG TAC TGG TAC GAG AAC TTC AAC ATC TCC ATT AGC AAC | 504 |
| | Ile Arg Tyr Trp Tyr Glu Asn Phe Asn Ile Ser Ile Ser Asn | |
| | 155 160 165 | |

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GTC ACA ACC AAA AAC AGC GGC AAC TAT TCC TGC TCA GGC CAG 546
Val Thr Thr Lys Asn Ser Gly Asn Tyr Ser Cys Ser Gly Gln
170 175

5 ATC CAG CAG AAA GGC TAC ACC TCT AAA GTC CTC AAC ATT ATT 588
Ile Gln Gln Lys Gly Tyr Thr Ser Lys Val Leu Asn Ile Ile
180 185 190

GTG NAA NNA GAG TAA GAATC 609
Val Xaa Xaa Glu
195

10 (2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 197 amino acids
(B) TYPE: amino acids
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: protein

(ix) FEATURES:
(A) NAME/KEY: Xaa = unknown amino acid
(B) LOCATION: 60, 195, 196

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

20 Met Pro Ala Ser Met Gly Gly Pro Ala Leu Trp Leu Ala
1 5 10

Leu Leu Leu Ser Ser Pro Gly Val Met Ser Ser Asp Thr Leu
15 20 25

25 Lys Pro Thr Val Ser Met Asn Pro Pro Trp Asn Thr Ile Leu
30 35 40

Lys Asp Asp Ser Val Thr Leu Thr Cys Thr Gly Asn Asn Ser
45 50 55

Leu Glu Val Xaa Ser Ala Val Trp Leu His Asn Asn Thr Thr
60 65 70

30 Leu Gln Glu Thr Thr Ser Arg Leu Asp Ile Asn Lys Ala Gln
75 80

Ile Gln Asp Ser Gly Glu Tyr Arg Cys Arg Glu Asn Arg Ser
85 90 95

35 Ile Leu Ser Asp Pro Val Tyr Leu Thr Val Phe Thr Glu Trp
100 105 110

Leu Ile Leu Gln Ala Ser Ala Asn Val Val Met Glu Gly Glu
115 120 125

Ser Phe Leu Ile Arg Cys His Ser Thr Lys Asn Leu Arg Leu
130 135 140

40 Thr Lys Val Thr Tyr Tyr Lys Asp Gly Ile Pro Ile Arg Tyr
145 150

Trp Tyr Glu Asn Phe Asn Ile Ser Ile Ser Asn Val Thr Thr
155 160 165

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Lys Asn Ser Gly Asn Tyr Ser Cys Ser Gly Gln Ile Gln Gln
 170 175 180

Lys Gly Tyr Thr Ser Lys Val Leu Asn Ile Ile Val Xaa Xaa
 185 190 195

5 Glu

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 609 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURES:

- (A) NAME/KEY: N = any nucleotide
 (B) LOCATION: 14, 15, 18

(ix) FEATURES:

- (A) NAME/KEY: M = A or C
 (B) LOCATION: 422

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

20	GAATTCCTTAC	TCTNNTTNCA	CAATAATGTT	GAGGACTTTA	GAGGTGTAGC	50
	CTTTCTGCTG	GATCTGGCCT	GAGCAGGAAT	AGTTGCCGCT	GTTTTTGTT	100
	GTGACGTTGC	TAATGGAGAT	GTTGAAGTTC	TCGTACCAGT	ACCTGATGGG	150
	GATGCCATCC	TTGTAGTAGG	TCACCTTTGT	GAGCCTCAAA	TTCTTCCAAC	200
	TATGGCACCT	GATGAGGAAG	CTCTCACCCT	CCATCACCAC	GTTGGCAGAG	250
25	GCTTGAAGGA	TCAGCCACTC	TGTGAAGACT	GTTAGGTACA	CAGGATCACT	300
	CAGGATGGAT	CTATTTTCCC	GACACCTGTA	CTCCCCACTG	TCCTGGATTT	350
	GGGCTTTATT	GATGTCCAAA	CGTGAAGTCG	TCTCTTGCAA	AGTAGTGTG	400
	TTGTGGAGCC	ACACAGCAGA	GMCGACTTCA	AGGGAGTTGT	TCCCAGTACA	450
	TGTAAGAGTC	ACACTGTCAT	CCTTCAATAT	TGTATTCCAT	GGCGGGTTCA	500
30	TGGACACTGT	AGGTTTCAAG	GTATCTGATG	ACATGACACC	TGGAGAGGAG	550
	AGCAGCAGCG	CTAGCCACAG	CAGGGCAGGG	CCTCCCATGG	AAGCAGGCAT	600
	ATTGGATCC					609

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 591 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURES:

- (A) NAME/KEY: Xaa = unknown amino acid
 (B) LOCATION: 60, 195, 196

(ix) FEATURES:

- (A) NAME/KEY: K = G or T
 (B) LOCATION: 179

(ix) FEATURES:

- (A) NAME/KEY: N = unknown nucleotide
 (B) LOCATION: 583, 586, 587

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(ix) FEATURES:

(A) NAME/KEY: CDS

(B) LOCATION: 1..591

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

5	ATG CCT GCT TCC ATG GGA GGC CCT GCC CTG CTG TGC CTA GCG	42
	Met Pro Ala Ser Met Gly Gly Pro Ala Leu Leu Trp Leu Ala	
	1 5 10	
	CTG CTG CTC TCC TCT CCA GGT GTC ATG TCA TCA GAT ACC TTG	84
10	Leu Leu Leu Ser Ser Pro Gly Val Met Ser Ser Asp Thr Leu	
	15 20 25	
	AAA CCT ACA GTG TCC ATG AAC CCG CCA TGG AAT ACA ATA TTG	126
	Lys Pro Thr Val Ser Met Asn Pro Pro Trp Asn Thr Ile Leu	
	30 35 40	
15	AAG GAT GAC AGT GTG ACT CTT ACA TGT ACT GGG AAC AAC TCC	168
	Lys Asp Asp Ser Val Thr Leu Thr Cys Thr Gly Asn Asn Ser	
	45 50 55	
	CTT GAA GTC GKC TCT GCT GTG TGG CTC CAC AAC AAC ACT ACT	210
	Leu Glu Val Xaa Ser Ala Val Trp Leu His Asn Asn Thr Thr	
	60 65 70	
20	TTG CAA GAG ACG ACT TCA CGT TTG GAC ATC AAT AAA GCC CAA	252
	Leu Gln Glu Thr Thr Ser Arg Leu Asp Ile Asn Lys Ala Gln	
	75 80	
	ATC CAG GAC AGT GGG GAG TAC AGG TGT CGG GAA AAT AGA TCC	294
25	Ile Gln Asp Ser Gly Glu Tyr Arg Cys Arg Glu Asn Arg Ser	
	85 90 95	
	ATC CTG AGT GAT CCT GTG TAC CTA ACA GTC TTC ACA GAG TGG	336
	Ile Leu Ser Asp Pro Val Tyr Leu Thr Val Phe Thr Glu Trp	
	100 105 110	
30	CTG ATC CTT CAA GCC TCT GCC AAC GTG GTG ATG GAG GGT GAG	378
	Leu Ile Leu Gln Ala Ser Ala Asn Val Val Met Glu Gly Glu	
	115 120 125	
	AGC TTC CTC ATC AGG TGC CAT AGT TGG AAG AAT TTG AGG CTC	420
	Ser Phe Leu Ile Arg Cys His Ser Trp Lys Asn Leu Arg Leu	
	130 135 140	
35	ACA AAG GTG ACC TAC TAC AAG GAT GGC ATC CCC ATC AGG TAC	462
	Thr Lys Val Thr Tyr Tyr Lys Asp Gly Ile Pro Ile Arg Tyr	
	145 150	
	TGG TAC GAG AAC TTC AAC ATC TCC ATT AGC AAC GTC ACA ACC	504
40	Trp Tyr Glu Asn Phe Asn Ile Ser Ile Ser Asn Val Thr Thr	
	155 160 165	
	AAA AAC AGC GGC AAC TAT TCC TGC TCA GGC CAG ATC CAG CAG	546
	Lys Asn Ser Gly Asn Tyr Ser Cys Ser Gly Gln Ile Gln Gln	
	170 175 180	
45	AAA GGC TAC ACC TCT AAA GTC CTC AAC ATT ATT GTG NAA NNA	588
	Lys Gly Tyr Thr Ser Lys Val Leu Asn Ile Ile Val Xaa Xaa	
	185 190 195	
	GAG	591
	Glu	

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(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 591 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cdna

(ix) FEATURES:
 (A) NAME/KEY: N = any nucleotide
 (B) LOCATION: 5, 6, 9

(ix) FEATURES:
 (A) NAME/KEY: M = A or C
 (B) LOCATION: 413

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

15	CTCTNNTTNC	ACAATAATGT	TGAGGACTTT	AGAGGTGTAG	CCTTTCTGCT	50
	GGATCTGGCC	TGAGCAGGAA	TAGTTGCCGC	TGTTTTTGGT	TGTGACGTTG	100
	CTAATGGAGA	TGTTGAAGTT	CTCGTACCAG	TACCTGATGG	GGATGCCATC	150
	CTTGTAGTAG	GTCACCTTTG	TGAGCCTCAA	ATTCTTCCAA	CTATGGCACC	200
	TGATGAGGAA	GCTCTCACCC	TCCATCACCA	CGTTGGCAGA	GGCTTGAAGG	250
20	ATCAGCCACT	CTGTGAAGAC	TGTTAGGTAC	ACAGGATCAC	TCAGGATGGA	300
	TCTATTTTCC	CGACACCTGT	ACTCCCCACT	GTCCTGGATT	TGGGCTTTAT	350
	TGATGTCCAA	ACGTGAAGTC	GTCTCTTGCA	AAGTAGTGTT	GTTGTGGAGC	400
	CACACAGCAG	AGMCGACTTC	AAGGGAGTTG	TTCCCAGTAC	ATGTAAGAGT	450
	CACACTGTCA	TCCTTCAATA	TTGTATTCCA	TGGCGGGTTC	ATGGACACTG	500
25	TAGGTTTCAA	GGTATCTGAT	GACATGACAC	CTGGAGAGGA	GAGCAGCAGC	550
	GCTAGCCACA	GCAGGGCAGG	GCCTCCCATG	GAAGCAGGCA	T	591

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 617 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cdna

(ix) FEATURES:
 (A) NAME/KEY: CDS
 (B) LOCATION: 10..606

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

40	GGATCCAAT	ATG	CCT	GCT	TCC	ATG	GGA	GGC	CCT	GCC	CTG	CTG	42	
		Met	Pro	Ala	Ser	Met	Gly	Gly	Pro	Ala	Leu	Leu		
		1				5						10		
	TGG	CTA	GCG	CTG	CTG	CTC	TCC	TCT	CCA	GGT	GTC	GTG	TCA	TCA
	Trp	Leu	Ala	Leu	Leu	Leu	Ser	Ser	Pro	Gly	Val	Val	Ser	Ser
		15					20					25		
45	GAT	ACC	TTG	AAA	CCT	ACA	GTG	TCC	ATG	AAC	CCG	CCA	TGG	AAT
	Asp	Thr	Leu	Lys	Pro	Thr	Val	Ser	Met	Asn	Pro	Pro	Trp	Asn
			30							35				
	ACA	ATA	TTG	AAG	GAT	GAC	AGT	GTG	ACT	CTT	ACA	TGT	ACT	GGG
	Thr	Ile	Leu	Lys	Asp	Asp	Ser	Val	Thr	Leu	Thr	Cys	Thr	Gly
	40					45					50			168

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AAC AAC TCC CTT GAA GTC GAC TCT GCT GTG TGG CTC CAC AAC 210
 Asn Asn Ser Leu Glu Val Asp Ser Ala Val Trp Leu His Asn
 55 60 65

AAC ACT ACT TTG CAA GAG ACG ACT TCA CGT TTG AAC ATC AAT 252
 5 Asn Thr Thr Leu Gln Glu Thr Thr Ser Arg Leu Asn Ile Asn
 70 75 80

AAA GCC CAA ATC CAG GAC AGT GGG GAG TAC AGG TGT CGG GAA 294
 Lys Ala Gln Ile Gln Asp Ser Gly Glu Tyr Arg Cys Arg Glu
 85 90 95

10 AAT AGA TCC ATC CTG AGT GAT CCT GTG TAC CTA ACA GTC TTC 336
 Asn Arg Ser Ile Leu Ser Asp Pro Val Tyr Leu Thr Val Phe
 100 105

ACA GAG TGG CTG ATC CTT CAA GCC TCT GCC AAC GTG GTG ATG 378
 15 Thr Glu Trp Leu Ile Leu Gln Ala Ser Ala Asn Val Val Met
 110 115 120

GAG GGT GAG AGC TTC CTC ATC AGG TGC CAT AGT TGG AAG AAT 420
 Glu Gly Glu Ser Phe Leu Ile Arg Cys His Ser Trp Lys Asn
 125 130 135

20 TTG AGG CTC ACA AAG GTG ACC TAC TAC AAG GAT GGC ATC CCC 462
 Leu Arg Leu Thr Lys Val Thr Tyr Tyr Lys Asp Gly Ile Pro
 140 145 150

ATC AGG TAC TGG TAC GAG AAC TTC AAC ATC TCC ATT AGC AAC 504
 Ile Arg Tyr Trp Tyr Glu Asn Phe Asn Ile Ser Ile Ser Asn
 155 160 165

25 GTC ACA ACC AAA AAC AGC GGC AAC TAT TCC TGC TCA GGC CAG 546
 Val Thr Thr Lys Asn Ser Gly Asn Tyr Ser Cys Ser Gly Gln
 170 175

ATC CAG CAG AAA GGC TAC ACC TCT AAA GTC CTC AAC ATT ATT 588
 30 Ile Gln Gln Lys Gly Tyr Thr Ser Lys Val Leu Asn Ile Ile
 180 185 190

GTG AAA AAG AGT AAG AAT TCTAAGAATT C 617
 Val Lys Lys Ser Lys Asn
 195

(2) INFORMATION FOR SEQ ID NO:14:

- 35 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 199 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- 40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:
- Met Pro Ala Ser Met Gly Gly Pro Ala Leu Leu Trp Leu Ala
 1 5 10
- Leu Leu Leu Ser Ser Pro Gly Val Val Ser Ser Asp Thr Leu
 15 20 25
- 45 Lys Pro Thr Val Ser Met Asn Pro Pro Trp Asn Thr Ile Leu
 30 35 40

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Lys Asp Asp Ser Val Thr Leu Thr Cys Thr Gly Asn Asn Ser
 45 50 55
 Leu Glu Val Asp Ser Ala Val Trp Leu His Asn Asn Thr Thr
 60 65 70
 5 Leu Gln Glu Thr Thr Ser Arg Leu Asn Ile Asn Lys Ala Gln
 75 80
 Ile Gln Asp Ser Gly Glu Tyr Arg Cys Arg Glu Asn Arg Ser
 85 90 95
 10 Ile Leu Ser Asp Pro Val Tyr Leu Thr Val Phe Thr Glu Trp
 100 105 110
 Leu Ile Leu Gln Ala Ser Ala Asn Val Val Met Glu Gly Glu
 115 120 125
 Ser Phe Leu Ile Arg Cys His Ser Trp Lys Asn Leu Arg Leu
 130 135 140
 15 Thr Lys Val Thr Tyr Tyr Lys Asp Gly Ile Pro Ile Arg Tyr
 145 150
 Trp Tyr Glu Asn Phe Asn Ile Ser Ile Ser Asn Val Thr Thr
 155 160 165
 20 Lys Asn Ser Gly Asn Tyr Ser Cys Ser Gly Gln Ile Gln Gln
 170 175 180
 Lys Gly Tyr Thr Ser Lys Val Leu Asn Ile Ile Val Lys Lys
 185 190 195
 Ser Lys Asn

(2) INFORMATION FOR SEQ ID NO:15:

- 25 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 617 nucleotides
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- 30 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

	GAATTCCTAG	AATTCTTACT	CTTTTTCACA	ATAATGTTGA	GGACTTTAGA	50
	GGTGTAGCCT	TTCTGCTGGA	TCTGGCCTGA	GCAGGAATAG	TTGCCGCTGT	100
	TTTGGTTGT	GACGTTGCTA	ATGGAGATGT	TGAAGTTCTC	GTACCAGTAC	150
35	CTGATGGGGA	TGCCATCCTT	G TAGTAGGTC	ACCTTTGTGA	GCCTCAAATT	200
	CTTCCAACTA	TGGCACCTGA	TGAGGAAGCT	CTCACCCCTCC	ATCACACCGT	250
	TGGCAGAGGC	TTGAAGGATC	AGCCACTCTG	TGAAGACTGT	TAGGTACACA	300
	GGATCACTCA	GGATGGATCT	ATTTTCCCGA	CACCTGTACT	CCCCACTGTC	350
	CTGGATTG	GCTTTATTGA	TGTTCAAACG	TGAAGTCGTC	TCTTGCAAAG	400
40	TAGTGTGTT	GTGGAGCCAC	ACAGCAGAGT	CGACTTCAAG	GGAGTTGTTT	450
	CCAGTACATG	TAAGAGTCAC	ACTGTCATCC	TTCAATATTG	TATTCCATGG	500
	CGGGTTCATG	GACACTGTAG	GTTTCAAGGT	ATCTGATGAC	ACGACACCTG	550
	GAGAGGAGAG	CAGCAGCGCT	AGCCACAGCA	GGGCAGGGCC	TCCCATGGAA	600
	GCAGGCATAT	TGGATCC				617

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(2) INFORMATION FOR SEQ ID NO:16

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 597 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURES:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..597

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

5	ATG CCT GCT TCC ATG GGA GGC CCT GCC CTG CTG TGG CTA GCG	42
	Met Pro Ala Ser Met Gly Gly Pro Ala Leu Leu Trp Leu Ala	
	1 5 10	
15	CTG CTG CTC TCC TCT CCA GGT GTC GTG TCA TCA GAT ACC TTG	84
	Leu Leu Leu Ser Ser Pro Gly Val Val Ser Ser Asp Thr Leu	
	15 20 25	
20	AAA CCT ACA GTG TCC ATG AAC CCG CCA TGG AAT ACA ATA TTG	126
	Lys Pro Thr Val Ser Met Asn Pro Pro Trp Asn Thr Ile Leu	
	30 35 40	
	AAG GAT GAC AGT GTG ACT CTT ACA TGT ACT GGG AAC AAC TCC	168
	Lys Asp Asp Ser Val Thr Leu Thr Cys Thr Gly Asn Asn Ser	
	45 50 55	
25	CTT GAA GTC GAC TCT GCT GTG TGG CTC CAC AAC AAC ACT ACT	210
	Leu Glu Val Asp Ser Ala Val Trp Leu His Asn Asn Thr Thr	
	60 65 70	
	TTG CAA GAG ACG ACT TCA CGT TTG AAC ATC AAT AAA GCC CAA	252
	Leu Gln Glu Thr Thr Ser Arg Leu Asn Ile Asn Lys Ala Gln	
	75 80	
30	ATC CAG GAC AGT GGG GAG TAC AGG TGT CGG GAA AAT AGA TCC	294
	Ile Gln Asp Ser Gly Glu Tyr Arg Cys Arg Glu Asn Arg Ser	
	85 90 95	
35	ATC CTG AGT GAT CCT GTG TAC CTA ACA GTC TTC ACA GAG TGG	336
	Ile Leu Ser Asp Pro Val Tyr Leu Thr Val Phe Thr Glu Trp	
	100 105 110	
	CTG ATC CTT CAA GCC TCT GCC AAC GTG GTG ATG GAG GGT GAG	378
	Leu Ile Leu Gln Ala Ser Ala Asn Val Val Met Glu Gly Glu	
	115 120 125	
40	AGC TTC CTC ATC AGG TGC CAT AGT TGG AAG AAT TTG AGG CTC	420
	Ser Phe Leu Ile Arg Cys His Ser Trp Lys Asn Leu Arg Leu	
	130 135 140	
	ACA AAG GTG ACC TAC TAC AAG GAT GGC ATC CCC ATC AGG TAC	462
	Thr Lys Val Thr Tyr Tyr Lys Asp Gly Ile Pro Ile Arg Tyr	
	145 150	
45	TGG TAC GAG AAC TTC AAC ATC TCC ATT AGC AAC GTC ACA ACC	504
	Trp Tyr Glu Asn Phe Asn Ile Ser Ile Ser Asn Val Thr Thr	
	155 160 165	

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AAA AAC AGC GGC AAC TAT TCC TGC TCA GGC CAG ATC CAG CAG 546
 Lys Asn Ser Gly Asn Tyr Ser Cys Ser Gly Gln Ile Gln Gln
 170 175 180

5 AAA GGC TAC ACC TCT AAA GTC CTC AAC ATT ATT GTG AAA AAG 588
 Lys Gly Tyr Thr Ser Lys Val Leu Asn Ile Ile Val Lys Lys
 185 190 195

AGT AAG AAT 597
 Ser Lys Asn

(2) INFORMATION FOR SEQ ID NO:17:

10 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 597 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ATTCTTACTC TTTTTCACAA TAATGTTGAG GACTTTAGAG GTGTAGCCTT 50
 TCTGCTGGAT CTGGCCTGAG CAGGAATAGT TGCCGCTGTT TTTGGTTGTG 100
 20 ACGTTGCTAA TGGAGATGTT GAAGTTCTCG TACCAGTACC TGATGGGGAT 150
 GCCATCCTTG TAGTAGGTCA CCTTTGTGAG CCTCAAATTC TTCCAACATAT 200
 GGCACCTGAT GAGGAAGCTC TCACCCTCCA TCACCACGTT GGCAGAGGCT 250
 TGAAGGATCA GCCACTCTGT GAAGACTGTT AGGTACACAG GATCACTCAG 300
 GATGGATCTA TTTTCCCGAC ACCTGTACTC CCCACTGTCC TGGATTTGGG 350
 25 CTTTATTGAT GTTCAAACGT GAAGTCGTCT CTTGCAAAGT AGTGTGTGTTG 400
 TGGAGCCACA CAGCAGAGTC GACTTCAAGG GAGTTGTTCC CAGTACATGT 450
 AAGAGTCACA CTGTCATCCT TCAATATTGT ATTCCATGGC GGGTTCATGG 500
 ACACTGTAGG TTTCAAGGTA TCTGATGACA CGACACCTGG AGAGGAGAGC 550
 AGCAGCGCTA GCCACAGCAG GGCAGGGCCT CCCATGGAAG CAGGCAT 597

(2) INFORMATION FOR SEQ ID NO:18:

30 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 17 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

35 (ix) FEATURES:
 (A) NAME/KEY: Xaa = any amino acid
 (B) LOCATION: 9, 14

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

40 Ser Asp Thr Leu Lys Pro Thr Val Xaa Met Asn Pro Pro Xaa
 1 5 10

Asn Leu Ile
 15

(2) INFORMATION FOR SEQ ID NO:19:

45 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 991 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: cDNA

(ix) FEATURES:

(A) NAME/KEY: CDS

(B) LOCATION: 35..796

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

	CTCCAGTCCA GTCGTACGTG GGGGCCACGA GGAG ATG CCT GCT	43
	Met Pro Ala	
	1	
10	TCC ATG GGA GGC CCT GCC CTG CTG TGG CTA GCG CTG CTG CTC	85
	Ser Met Gly Gly Pro Ala Leu Leu Trp Leu Ala Leu Leu Leu	
	5 10 15	
	TCC TCT CCA GGT GTC ATG TCA TCA GAT ACC TTG AAA CCT ACA	127
	Ser Ser Pro Gly Val Met Ser Ser Asp Thr Leu Lys Pro Thr	
	20 25 30	
15	GTG TCC ATG AAC CCG CCA TGG AAT ACA ATA TTG AAG GAT GAC	169
	Val Ser Met Asn Pro Pro Trp Asn Thr Ile Leu Lys Asp Asp	
	35 40 45	
20	AGT GTG ACT CTT ACA TGT ACT GGG AAC AAC TCC CTT GAA GTC	211
	Ser Val Thr Leu Thr Cys Thr Gly Asn Asn Ser Leu Glu Val	
	50 55	
	GAC TCT GCT GTG TGG CTC CAC AAC AAC ACT ACT TTG CAA GAG	253
	Asp Ser Ala Val Trp Leu His Asn Asn Thr Thr Leu Gln Glu	
	60 65 70	
25	ACG ACT TCA CGT TTG GAC ATC AAT AAA GCC CAA ATC CAG GAC	295
	Thr Thr Ser Arg Leu Asp Ile Asn Lys Ala Gln Ile Gln Asp	
	75 80 85	
	AGT GGG GAG TAC AGG TGT CGG GAA AAT AGA TCC ATC CTG AGT	337
	Ser Gly Glu Tyr Arg Cys Arg Glu Asn Arg Ser Ile Leu Ser	
	90 95 100	
30	GAT CCT GTG TAC CTA ACA GTC TTC ACA GAG TGG CTG ATC CTT	379
	Asp Pro Val Tyr Leu Thr Val Phe Thr Glu Trp Leu Ile Leu	
	105 110 115	
35	CAA GCC TCT GCC AAC GTG GTG ATG GAG GGT GAG AGC TTC CTC	421
	Gln Ala Ser Ala Asn Val Val Met Glu Gly Glu Ser Phe Leu	
	120 125	
	ATC AGG TGC CAT AGT TGG AAG AAT TTG AGG CTC ACA AAG GTG	463
	Ile Arg Cys His Ser Trp Lys Asn Leu Arg Leu Thr Lys Val	
	130 135 140	
40	ACC TAC TAC AAG GAT GGC ATC CCC ATC AGG TAC TGG TAC GAG	505
	Thr Tyr Tyr Lys Asp Gly Ile Pro Ile Arg Tyr Trp Tyr Glu	
	145 150 155	
	AAC TTC AAC ATC TCC ATT AGC AAC GTC ACA ACC AAA AAC AGC	437
	Asn Phe Asn Ile Ser Ile Ser Asn Val Thr Thr Lys Asn Ser	
	160 165 170	
45	GGC AAC TAT TCC TGC TCA GGC CAG ATC CAG CAG AAA GGC TAC	589
	Gly Asn Tyr Ser Cys Ser Gly Gln Ile Gln Gln Lys Gly Tyr	
	175 180 185	

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ACC TCT AAA GTC CTC AAC ATT ATT GTG AAA AAA GAG CCC ACC 631
 Thr Ser Lys Val Leu Asn Ile Ile Val Lys Lys Glu Pro Thr
 190 195

5 AAG CAA AAC AAG TAC TCC GGG CTA CAA TTC CTG ATC CCG TTG 673
 Lys Gln Asn Lys Tyr Ser Gly Leu Gln Phe Leu Ile Pro Leu
 200 205 210

GTG GTG GTG ATT CTG TTT GCT GTG GAC ACA GGA CTG TTT ATC 715
 Val Val Val Ile Leu Phe Ala Val Asp Thr Gly Leu Phe Ile
 215 220 225

10 TCG ACC AAG CAG CAG TTG ACA GTG CTC TTG CAG ATT AAG AGG 757
 Ser Thr Lys Gln Gln Leu Thr Val Leu Leu Gln Ile Lys Arg
 230 235 240

ACC AGG AAG AAC AAA AAG CCA GAA CCC GGA AAG AAC TGA 796
 Thr Arg Lys Asn Lys Lys Pro Glu Pro Gly Lys Asn
 15 245 250

TGCCGCTGCT TAAGAAACAT CAGCATCAGC AATCGCTTCT CCATCGTCAG 846
 ACGCAGCTCA CGATGCACAC GCGAAGGTCT GCAGTCATGG CTTTGCAGAA 896
 CTGCTTCATT CAACCAACTC AAAGTGATTA AGTGGCATGT GATAGTAGGT 946
 GCTCAATAAA CGGCAGTTAG ATAAATAAAA AAAAAAAAAA AAAAA 991

20 (2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 253 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Met Pro Ala Ser Met Gly Gly Pro Ala Leu Leu Trp Leu Ala
 1 5 10

Leu Leu Leu Ser Ser Pro Gly Val Met Ser Ser Asp Thr Leu
 15 20 25

Lys Pro Thr Val Ser Met Asn Pro Pro Trp Asn Thr Ile Leu
 30 35 40

Lys Asp Asp Ser Val Thr Leu Thr Cys Thr Gly Asn Asn Ser
 45 50 55

35 Leu Glu Val Asp Ser Ala Val Trp Leu His Asn Asn Thr Thr
 60 65 70

Leu Gln Glu Thr Thr Ser Arg Leu Asp Ile Asn Lys Ala Gln
 75 80

40 Ile Gln Asp Ser Gly Glu Tyr Arg Cys Arg Glu Asn Arg Ser
 85 90 95

Ile Leu Ser Asp Pro Val Tyr Leu Thr Val Phe Thr Glu Trp
 100 105 110

Leu Ile Leu Gln Ala Ser Ala Asn Val Val Met Glu Gly Glu
 115 120 125

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Ser Phe Leu Ile Arg Cys His Ser Trp Lys Asn Leu Arg Leu
 130 135 140

Thr Lys Val Thr Tyr Tyr Lys Asp Gly Ile Pro Ile Arg Tyr
 145 150

5 Trp Tyr Glu Asn Phe Asn Ile Ser Ile Ser Asn Val Thr Thr
 155 160 165

Lys Asn Ser Gly Asn Tyr Ser Cys Ser Gly Gln Ile Gln Gln
 170 175 180

10 Lys Gly Tyr Thr Ser Lys Val Leu Asn Ile Ile Val Lys Lys
 185 190 195

Glu Pro Thr Lys Gln Asn Lys Tyr Ser Gly Leu Gln Phe Leu
 200 205 210

Ile Pro Leu Val Val Val Ile Leu Phe Ala Val Asp Thr Gly
 215 220

15 Leu Phe Ile Ser Thr Lys Gln Gln Leu Thr Val Leu Leu Gln
 225 230 235

Ile Lys Arg Thr Arg Lys Asn Lys Lys Pro Glu Pro Gly Lys
 240 245 250

Asn

20 (2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 991 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 25 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

	TTTTTTTTTT	TTTTTTTTTT	TTTATCTAAC	TGCCGTTTAT	TGAGCACCTA	50
	CTATCACATG	CCACTTAATC	AGTTTGAGTT	GGTTGAATGA	AGCAGTTCTG	100
30	CAAAGCCATG	ACTGCAGACC	TTCCCGTGTG	CATCGTGAGC	TGCGTCTGAC	150
	GATGGAGAAG	CGATTGCTGA	TGCTGATGTT	TCTTAAGCAG	CGGCATCAGT	200
	TCTTTCCGGG	TTCTGGCTTT	TTGTTCTTCC	TGGTCCTCTT	AATCTGCAAG	250
	AGCACTGTCA	ACTGCTGCTT	GGTCGAGATA	AACAGTCCTG	TGTCCACAGC	300
	AAACAGAATC	ACCACCACCA	ACGGGATCAG	GAATTGTAGC	CCGGAGTACT	350
35	TGTTTTGCTT	GGTGGGCTCT	TTTTTCACAA	TAATGTTGAG	GACTTTAGAG	400
	GTGTAGCCTT	TCTGCTGGAT	CTGGCCTGAG	CAGGAATAGT	TGCCGCTGTT	450
	TTTGTTTGTT	ACGTTGCTAA	TGGAGATGTT	GAAGTTCTCG	TACCAGTACC	500
	TGATGGGGAT	GCCATCCTTG	TAGTAGGTCA	CCTTTGTGAG	CCTCAAATTC	550
	TTCCAACAT	GGCACCTGAT	GAGGAAGCTC	TCACCCTCCA	TCACCACGTT	600
40	GGCAGAGGCT	TGAAGGATCA	GCCACTCTGT	GAAGACTGTT	AGGTACACAG	650
	GATCACTCAG	GATGGATCTA	TTTTCCCGAA	TCTGTACTC	CCCACGTGCC	700
	TGGATTTGGG	CTTTATTGAT	GTCCAAACGT	GAAGTCGTCT	CTTGCAAAGT	750
	AGTGTGTGTT	TGGAGCCACA	CAGCAGAGTC	GACTTCAAGG	GAGTTGTTCC	800
	CAGTACATGT	AAGAGTCACA	CTGTCATCCT	TCAATATTGT	ATTCCATGGC	850
45	GGGTTTCATG	AACTGTAGG	TTTCAAGGTA	TCTGATGACA	TGACACCTGG	900
	AGAGGAGAGC	AGCAGCGCTA	GCCACAGCAG	GGCAGGGCCT	CCCATGGAAG	950
	CAGGCATCTC	CTCGTGGCCC	CCACGTACGA	CTGGACTGGA	G	991

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(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 759 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURES:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..759

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

5	ATG CCT GCT TCC ATG GGA GGC CCT GCC CTG CTG TGG CTA GCG	42
	Met Pro Ala Ser Met Gly Gly Pro Ala Leu Leu Trp Leu Ala	
	1 5 10	
15	CTG CTG CTC TCC TCT CCA GGT GTC ATG TCA TCA GAT ACC TTG	84
	Leu Leu Leu Ser Ser Pro Gly Val Met Ser Ser Asp Thr Leu	
	15 20 25	
20	AAA CCT ACA GTG TCC ATG AAC CCG CCA TGG AAT ACA ATA TTG	126
	Lys Pro Thr Val Ser Met Asn Pro Pro Trp Asn Thr Ile Leu	
	30 35 40	
	AAG GAT GAC AGT GTG ACT CTT ACA TGT ACT GGG AAC AAC TCC	168
	Lys Asp Asp Ser Val Thr Leu Thr Cys Thr Gly Asn Asn Ser	
	45 50 55	
25	CTT GAA GTC GAC TCT GCT GTG TGG CTC CAC AAC AAC ACT ACT	210
	Leu Glu Val Asp Ser Ala Val Trp Leu His Asn Asn Thr Thr	
	60 65 70	
	TTG CAA GAG ACG ACT TCA CGT TTG GAC ATC AAT AAA GCC CAA	252
	Leu Gln Glu Thr Thr Ser Arg Leu Asp Ile Asn Lys Ala Gln	
	75 80	
30	ATC CAG GAC AGT GGG GAG TAC AGG TGT CGG GAA AAT AGA TCC	294
	Ile Gln Asp Ser Gly Glu Tyr Arg Cys Arg Glu Asn Arg Ser	
	85 90 95	
35	ATC CTG AGT GAT CCT GTG TAC CTA ACA GTC TTC ACA GAG TGG	336
	Ile Leu Ser Asp Pro Val Tyr Leu Thr Val Phe Thr Glu Trp	
	100 105 110	
	CTG ATC CTT CAA GCC TCT GCC AAC GTG GTG ATG GAG GGT GAG	378
	Leu Ile Leu Gln Ala Ser Ala Asn Val Val Met Glu Gly Glu	
	115 120 125	
40	AGC TTC CTC ATC AGG TGC CAT AGT TGG AAG AAT TTG AGG CTC	420
	Ser Phe Leu Ile Arg Cys His Ser Trp Lys Asn Leu Arg Leu	
	130 135 140	
	ACA AAG GTG ACC TAC TAC AAG GAT GGC ATC CCC ATC AGG TAC	462
	Thr Lys Val Thr Tyr Tyr Lys Asp Gly Ile Pro Ile Arg Tyr	
	145 150	
45	TGG TAC GAG AAC TTC AAC ATC TCC ATT AGC AAC GTC ACA ACC	504
	Trp Tyr Glu Asn Phe Asn Ile Ser Ile Ser Asn Val Thr Thr	
	155 160 165	

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	AAA AAC AGC GGC AAC TAT TCC TGC TCA GGC CAG ATC CAG CAG	546
	Lys Asn Ser Gly Asn Tyr Ser Cys Ser Gly Gln Ile Gln Gln	
	170 175 180	
5	AAA GGC TAC ACC TCT AAA GTC CTC AAC ATT ATT GTG AAA AAA	588
	Lys Gly Tyr Thr Ser Lys Val Leu Asn Ile Ile Val Lys Lys	
	185 190 195	
	GAG CCC ACC AAG CAA AAC AAG TAC TCC GGG CTA CAA TTC CTG	630
	Glu Pro Thr Lys Gln Asn Lys Tyr Ser Gly Leu Gln Phe Leu	
	200 205 210	
10	ATC CCG TTG GTG GTG GTG ATT CTG TTT GCT GTG GAC ACA GGA	672
	Ile Pro Leu Val Val Val Ile Leu Phe Ala Val Asp Thr Gly	
	215 220	
	CTG TTT ATC TCG ACC AAG CAG CAG TTG ACA GTG CTC TTG CAG	714
	Leu Phe Ile Ser Thr Lys Gln Gln Leu Thr Val Leu Leu Gln	
15	225 230 235	
	ATT AAG AGG ACC AGG AAG AAC AAA AAG CCA GAA CCC GGA AAG	756
	Ile Lys Arg Thr Arg Lys Asn Lys Lys Pro Glu Pro Gly Lys	
	240 245 250	
20	AAC	759
	Asn	
	(2) INFORMATION FOR SEQ ID NO:23:	
	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 759 nucleotides	
25	(B) TYPE: nucleic acid	
	(C) STRANDEDNESS: single	
	(D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: cDNA	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:	
30	GTCTCTTCCG GGTTCTGGCT TTTTGTCTCTT CCTGGTCCTC TTAATCTGCA	50
	AGAGCACTGT CAACTGCTGC TTGGTCGAGA TAAACAGTCC TGTGTCCACA	100
	GCAAACAGAA TCACCACCAC CAACGGGATC AGGAATTGTA GCCCGGAGTA	150
	CTTGTTTTCG TTGGTGGGCT CTTTTTTCAC AATAATGTTG AGGACTTTAG	200
	AGGTGTAGCC TTTCTGCTGG ATCTGGCCTG AGCAGGAATA GTTGCCGCTG	250
	TTTTTGTTG TGACGTTGCT AATGGAGATG TTGAAGTTCT CGTACCAGTA	300
35	CCTGATGGGG ATGCCATCCT TGTAGTAGGT CACCTTTGTG AGCCTCAAAT	350
	TCTTCCAAC ATGGCACCTG ATGAGGAAGC TCTCACCTC CATCACACG	400
	TTGGCAGAGG CTTGAAGGAT CAGCCACTCT GTGAAGACTG TTAGGTACAC	450
	AGGATCACTC AGGATGGATC TATTTTCCCG ACACCTGTAC TCCCCACTGT	500
	CCTGGATTG GGCTTTATTG ATGTCCAAAC GTGAAGTCGT CTCTTGCAA	550
40	GTAGTGTGT TGTGGAGCCA CACAGCAGAG TCGACTTCAA GGGAGTTGTT	600
	CCCAGTACAT GTAAGAGTCA CACTGTCATC CTTCAATATT GTATTCCATG	650
	GCGGGTTCAT GGACACTGTA GGTTCACAGG TATCTGATGA CATGACACCT	700
	GGAGAGGAGA GCAGCAGCGC TAGCCACAGC AGGGCAGGGC CTCCCATGGA	750
	AGCAGGCAT	759

45 (2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 229 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: protein

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Ser Asp Thr Leu Lys Pro Thr Val Ser Met Asn Pro Pro Trp
 1 5 10
 5 Asn Thr Ile Leu Lys Asp Asp Ser Val Thr Leu Thr Cys Thr
 15 20 25
 Gly Asn Asn Ser Leu Glu Val Asp Ser Ala Val Trp Leu His
 30 35 40
 Asn Asn Thr Thr Leu Gln Glu Thr Thr Ser Arg Leu Asp Ile
 45 50 55
 10 Asn Lys Ala Gln Ile Gln Asp Ser Gly Glu Tyr Arg Cys Arg
 60 65 70
 Glu Asn Arg Ser Ile Leu Ser Asp Pro Val Tyr Leu Thr Val
 75 80
 15 Phe Thr Glu Trp Leu Ile Leu Gln Ala Ser Ala Asn Val Val
 85 90 95
 Met Glu Gly Glu Ser Phe Leu Ile Arg Cys His Ser Trp Lys
 100 105 110
 Asn Leu Arg Leu Thr Lys Val Thr Tyr Tyr Lys Asp Gly Ile
 115 120 125
 20 Pro Ile Arg Tyr Trp Tyr Glu Asn Phe Asn Ile Ser Ile Ser
 130 135 140
 Asn Val Thr Thr Lys Asn Ser Gly Asn Tyr Ser Cys Ser Gly
 145 150
 25 Gln Ile Gln Gln Lys Gly Tyr Thr Ser Lys Val Leu Asn Ile
 155 160 165
 Ile Val Lys Lys Glu Pro Thr Lys Gln Asn Lys Tyr Ser Gly
 170 175 180
 Leu Gln Phe Leu Ile Pro Leu Val Val Val Ile Leu Phe Ala
 185 190 195
 30 Val Asp Thr Gly Leu Phe Ile Ser Thr Lys Gln Gln Leu Thr
 200 205 210
 Val Leu Leu Gln Ile Lys Arg Thr Arg Lys Asn Lys Lys Pro
 215 220
 35 Glu Pro Gly Lys Asn
 225

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 33 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: primer

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GCGAAGATCT ATAAATATGC CTGCTTCCAT GGG

33

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GCAGGAATTC TTACTCTTTT TTCACAATAA TGT

33

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 591 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURES:

(A) NAME/KEY: CDS
 (B) LOCATION: 1..591

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

ATG CCT GCT TCC ATG GGA GGC CCT GCC CTG CTG TGG CTA GCG
 Met Pro Ala Ser Met Gly Gly Pro Ala Leu Leu Trp Leu Ala

42

25 1 5 10

CTG CTG CTC TCC TCT CCA GGT GTC ATG TCA TCA GAT ACC TTG
 Leu Leu Leu Ser Ser Pro Gly Val Met Ser Ser Asp Thr Leu

84

15 20 25

AAA CCT ACA GTG TCC ATG AAC CCG CCA TGG AAT ACA ATA TTG
 Lys Pro Thr Val Ser Met Asn Pro Pro Trp Asn Thr Ile Leu

126

30 35 40

AAG GAT GAC AGT GTG ACT CTT ACA TGT ACT GGG AAC AAC TCC
 Lys Asp Asp Ser Val Thr Leu Thr Cys Thr Gly Asn Asn Ser

168

45 50 55

CTT GAA GTC GAC TCT GCT GTG TGG CTC CAC AAC AAC ACT ACT
 Leu Glu Val Asp Ser Ala Val Trp Leu His Asn Asn Thr Thr

210

60 65 70

TTG CAA GAG ACG ACT TCA CTT TTG GAC ATC AAT AAA GCC CAA
 Leu Gln Glu Thr Thr Ser Arg Leu Asp Ile Asn Lys Ala Gln

252

40 75 80

ATC CAG GAC AGT GGG GAG TAC AGG TGT CGG GAA AAT AGA TCC
 Ile Gln Asp Ser Gly Glu Tyr Arg Cys Arg Glu Asn Arg Ser

294

85 90 95

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	ATC	CTG	AGT	GAT	CCT	GTG	TAC	CTA	ACA	GTC	TTC	ACA	GAG	TGG	336
	Ile	Leu	Ser	Asp	Pro	Val	Tyr	Leu	Thr	Val	Phe	Thr	Glu	Trp	
	100						105					110			
5	CTG	ATC	CTT	CAA	GCC	TCT	GCC	AAC	GTG	GTG	ATG	GAG	GGT	GAG	378
	Leu	Ile	Leu	Gln	Ala	Ser	Ala	Asn	Val	Val	Met	Glu	Gly	Glu	
			115					120					125		
	AGC	TTC	CTC	ATC	AGG	TGC	CAT	AGT	TGG	AAG	AAT	TTG	AGG	CTC	420
	Ser	Phe	Leu	Ile	Arg	Cys	His	Ser	Trp	Lys	Asn	Leu	Arg	Leu	
				130					135					140	
10	ACA	AAG	GTG	ACC	TAC	TAC	AAG	GAT	GGC	ATC	CCC	ATC	AGG	TAC	462
	Thr	Lys	Val	Thr	Tyr	Tyr	Lys	Asp	Gly	Ile	Pro	Ile	Arg	Tyr	
					145					150					
15	TGG	TAC	GAG	AAC	TTC	AAC	ATC	TCC	ATT	AGC	AAC	GTC	ACA	ACC	504
	Trp	Tyr	Glu	Asn	Phe	Asn	Ile	Ser	Ile	Ser	Asn	Val	Thr	Thr	
	155					160					165				
	AAA	AAC	AGC	GGC	AAC	TAT	TCC	TGC	TCA	GGC	CAG	ATC	CAG	CAG	546
	Lys	Asn	Ser	Gly	Asn	Tyr	Ser	Cys	Ser	Gly	Gln	Ile	Gln	Gln	
		170					175					180			
20	AAA	GGC	TAC	ACC	TCT	AAA	GTC	CTC	AAC	ATT	ATT	GTG	AAA	AAA	588
	Lys	Gly	Tyr	Thr	Ser	Lys	Val	Leu	Asn	Ile	Ile	Val	Lys	Lys	
			185					190					195		
	GAG														591
	Glu														

(2) INFORMATION FOR SEQ ID NO:28:

- 25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 196 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- 30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:
- Met Pro Ala Ser Met Gly Gly Pro Ala Leu Leu Trp Leu Ala
 1 5 10
- Leu Leu Leu Ser Ser Pro Gly Val Met Ser Ser Asp Thr Leu
 15 20 25
- 35 Lys Pro Thr Val Ser Met Asn Pro Pro Trp Asn Thr Ile Leu
 30 35 40
- Lys Asp Asp Ser Val Thr Leu Thr Cys Thr Gly Asn Asn Ser
 45 50 55
- 40 Leu Glu Val Asp Ser Ala Val Trp Leu His Asn Asn Thr Thr
 60 65
- Leu Gln Glu Thr Thr Ser Arg Leu Asp Ile Asn Lys Ala Gln
 70 75 80
- Ile Gln Asp Ser Gly Glu Tyr Arg Cys Arg Glu Asn Arg Ser
 85 90 95

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Ile Leu Ser Asp Pro Val Tyr Leu Thr Val Phe Thr Glu Trp
 100 105 110

Leu Ile Leu Gln Ala Ser Ala Asn Val Val Met Glu Gly Glu
 115 120 125

5 Ser Phe Leu Ile Arg Cys His Ser Trp Lys Asn Leu Arg Leu
 130 135

Thr Lys Val Thr Tyr Tyr Lys Asp Gly Ile Pro Ile Arg Tyr
 140 145 150

10 Trp Tyr Glu Asn Phe Asn Ile Ser Ile Ser Asn Val Thr Thr
 155 160 165

Lys Asn Ser Gly Asn Tyr Ser Cys Ser Gly Gln Ile Gln Gln
 170 175 180

Lys Gly Tyr Thr Ser Lys Val Leu Asn Ile Ile Val Lys Lys
 185 190 195

15 Glu

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 591 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

25 CTCTTTTTC ACAATAATGT TGAGGACTTT AGAGGTGTAG CCTTCTGCT 50
 GGATCTGGCC TGAGCAGGAA TAGTTGCCGC TGTTTTTGGT TGTGACGTTG 100
 CTAATGGAGA TGTTGAAGTT CTCGTACCAG TACCTGATGG GGATGCCATC 150
 CTTGTAGTAG GTCACCTTTG TGAGCCTCAA ATTCTTCCAA CTATGGCACC 200
 TGATGAGGAA GCTCTCACCC TCCATCACCA CGTTGGCAGA GGCTTGAAGG 250
 ATCAGCCACT CTGTGAAGAC TGTTAGGTAC ACAGGATCAC TCAGGATGGA 300
 30 TCTATTTTCC CGACACCTGT ACTCCCCACT GTCCTGGATT TGGGCTTTAT 350
 TGATGTCCAA ACGTGAAGTC GTCTCTTGCA AAGTAGTGTT GTTGTGGAGC 400
 CACACAGCAG AGTCGACTTC AAGGGAGTTG TTCCAGTAC ATGTAAGAGT 450
 CACACTGTCA TCCTTCAATA TTGTATTCCA TGGCGGGTTC ATGGACACTG 500
 TAGGTTTCAA GGTATCTGAT GACATGACAC CTGGAGAGGA GAGCAGCAGC 550
 35 GCTAGCCACA GCAGGCAGG GCCTCCCATG GAAGCAGGCA T 591

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 687 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

40

(ii) MOLECULE TYPE: cDNA

(ix) FEATURES:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..687

45

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

	TCA	GAT	ACC	TTG	AAA	CCT	ACA	GTG	TCC	ATG	AAC	CCG	CCA	TGG	42
	Ser	Asp	Thr	Leu	Lys	Pro	Thr	Val	Ser	Met	Asn	Pro	Pro	Trp	
	1				5					10					
5	AAT	ACA	ATA	TTG	AAG	GAT	GAC	AGT	GTG	ACT	CTT	ACA	TGT	ACT	84
	Asn	Thr	Ile	Leu	Lys	Asp	Asp	Ser	Val	Thr	Leu	Thr	Cys	Thr	
	15				20					25					
10	GGG	AAC	AAC	TCC	CTT	GAA	GTC	GAC	TCT	GCT	GTG	TGG	CTC	CAC	126
	Gly	Asn	Asn	Ser	Leu	Glu	Val	Asp	Ser	Ala	Val	Trp	Leu	His	
	30					35					40				
	AAC	AAC	ACT	ACT	TTG	CAA	GAG	ACG	ACT	TCA	CGT	TTG	GAC	ATC	168
	Asn	Asn	Thr	Thr	Leu	Gln	Glu	Thr	Thr	Ser	Arg	Leu	Asp	Ile	
			45					50					55		
15	AAT	AAA	GCC	CAA	ATC	CAG	GAC	AGT	GGG	GAG	TAC	AGG	TGT	CGG	210
	Asn	Lys	Ala	Gln	Ile	Gln	Asp	Ser	Gly	Glu	Tyr	Arg	Cys	Arg	
			60						65				70		
	GAA	AAT	AGA	TCC	ATC	CTG	AGT	GAT	CCT	GTG	TAC	CTA	ACA	GTC	252
	Glu	Asn	Arg	Ser	Ile	Leu	Ser	Asp	Pro	Val	Tyr	Leu	Thr	Val	
					75					80					
20	TTC	ACA	GAG	TGG	CTG	ATC	CTT	CAA	GCC	TCT	GCC	AAC	GTG	GTG	294
	Phe	Thr	Glu	Trp	Leu	Ile	Leu	Gln	Ala	Ser	Ala	Asn	Val	Val	
	85					90					95				
25	ATG	GAG	GGT	GAG	AGC	TTC	CTC	ATC	AGG	TGC	CAT	AGT	TGG	AAG	336
	Met	Glu	Gly	Glu	Ser	Phe	Leu	Ile	Arg	Cys	His	Ser	Trp	Lys	
	100					105						110			
	AAT	TTG	AGG	CTC	ACA	AAG	GTG	ACC	TAC	TAC	AAG	GAT	GGC	ATC	378
	Asn	Leu	Arg	Leu	Thr	Lys	Val	Thr	Tyr	Tyr	Lys	Asp	Gly	Ile	
			115					120					125		
30	CCC	ATC	AGG	TAC	TGG	TAC	GAG	AAC	TTC	AAC	ATC	TCC	ATT	AGC	420
	Pro	Ile	Arg	Tyr	Trp	Tyr	Glu	Asn	Phe	Asn	Ile	Ser	Ile	Ser	
				130					135					140	
	AAC	GTC	ACA	ACC	AAA	AAC	AGC	GGC	AAC	TAT	TCC	TGC	TCA	GGC	462
	Asn	Val	Thr	Thr	Lys	Asn	Ser	Gly	Asn	Tyr	Ser	Cys	Ser	Gly	
					145					150					
35	CAG	ATC	CAG	CAG	AAA	GGC	TAC	ACC	TCT	AAA	GTC	CTC	AAC	ATT	504
	Gln	Ile	Gln	Gln	Lys	Gly	Tyr	Thr	Ser	Lys	Val	Leu	Asn	Ile	
	155					160					165				
40	ATT	GTG	AAA	AAA	GAG	CCC	ACC	AAG	CAA	AAC	AAG	TAC	TCC	GGG	546
	Ile	Val	Lys	Lys	Glu	Pro	Thr	Lys	Gln	Asn	Lys	Tyr	Ser	Gly	
	170					175						180			
	CTA	CAA	TTC	CTG	ATC	CCG	TTG	GTG	GTG	GTG	..T.	CTG	TTT	GCT	588
	Leu	Gln	Phe	Leu	Ile	Pro	Leu	Val	Val	Val	Ile	Leu	Phe	Ala	
			185					190					195		
45	GTG	GAC	ACA	GGA	CTG	TTT	ATC	TCG	ACC	AAG	CAG	CAG	TTG	ACA	630
	Val	Asp	Thr	Gly	Leu	Phe	Ile	Ser	Thr	Lys	Gln	Gln	Leu	Thr	
				200					205					210	

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GTG CTC TTG CAG ATT AAG AGG ACC AGG AAG AAC AAA AAG CCA 672
 Val Leu Leu Gln Ile Lys Arg Thr Arg Lys Asn Lys Lys Pro
 215 220

5 GAA CCC GGA AAG AAC 687
 Glu Pro Gly Lys Asn
 225

(2) INFORMATION FOR SEQ ID NO:31:

10 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 173 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

15 Ser Asp Thr Leu Lys Pro Thr Val Ser Met Asn Pro Pro Trp
 1 5 10
 Asn Thr Ile Leu Lys Asp Asp Ser Val Thr Leu Thr Cys Thr
 15 20 25
 Gly Asn Asn Ser Leu Glu Val Asp Ser Ala Val Trp Leu His
 30 35 40
 20 Asn Asn Thr Thr Leu Gln Glu Thr Thr Ser Arg Leu Asp Ile
 45 50 55
 Asn Lys Ala Gln Ile Gln Asp Ser Gly Glu Tyr Arg Cys Arg
 60 65 70
 25 Glu Asn Arg Ser Ile Leu Ser Asp Pro Val Tyr Leu Thr Val
 75 80
 Phe Thr Glu Trp Leu Ile Leu Gln Ala Ser Ala Asn Val Val
 85 90 95
 Met Glu Gly Glu Ser Phe Leu Ile Arg Cys His Ser Trp Lys
 100 105 110
 30 Asn Leu Arg Leu Thr Lys Val Thr Tyr Tyr Lys Asp Gly Ile
 115 120 125
 Pro Ile Arg Tyr Trp Tyr Glu Asn Phe Asn Ile Ser Ile Ser
 130 135 140
 35 Asn Val Thr Thr Lys Asn Ser Gly Asn Tyr Ser Cys Ser Gly
 145 150
 Gln Ile Gln Gln Lys Gly Tyr Thr Ser Lys Val Leu Asn Ile
 155 160 165
 Ile Val Lys Lys Glu
 170

While various embodiments of the present invention have been described in detail, it is apparent that modifications and adaptations of those embodiments will occur to those skilled in the art. It is to be expressly understood, however, that such modifications and adaptations are within the scope of the present invention, as set forth

5 in the following claims.

What is claimed is:

1. A method to detect canine IgE comprising:
 - (a) contacting an isolated canine $Fc_\epsilon R$ receptor ($Fc_\epsilon R$) molecule with a putative canine IgE-containing composition under conditions suitable for formation of a $Fc_\epsilon R$ molecule:IgE complex; and
 - (b) determining the presence of IgE by detecting said $Fc_\epsilon R$ molecule:IgE complex, the presence of said $Fc_\epsilon R$ molecule:IgE complex indicating the presence of IgE.
2. A kit for detecting IgE comprising a canine $Fc_\epsilon R$ molecule and a means for detecting canine IgE.
3. A method to detect canine flea allergy dermatitis comprising:
 - (a) immobilizing a flea allergen on a substrate;
 - (b) contacting said flea allergen with a putative canine IgE-containing composition under conditions suitable for formation of an antigen:IgE complex bound to said substrate;
 - (c) removing non-bound material from said substrate under conditions that retain antigen:IgE complex binding to said substrate; and
 - (d) detecting the presence of said antigen:IgE complex by contacting said antigen:IgE complex with a canine $Fc_\epsilon R$ molecule.
4. A kit for detecting flea allergy dermatitis comprising a canine $Fc_\epsilon R$ receptor molecule and a flea allergen.
5. The invention of Claims 1, 2, 3 or 4, wherein said $Fc_\epsilon R$ molecule comprises at least a portion of a $Fc_\epsilon R$ alpha chain that binds to canine IgE.
6. The invention of Claims 1, 2, 3 or 4, wherein said $Fc_\epsilon R$ molecule comprises a protein selected from the group consisting of $PcFc_\epsilon R\alpha 1_{197}$, $PcFc_\epsilon R\alpha 2_{197}$, $PcFc_\epsilon R\alpha 3_{199}$, $PcFc_\epsilon R\alpha 4_{253}$, $PcFc_\epsilon R\alpha 4_{229}$, $PcFc_\epsilon R\alpha 4_{173}$ and $PcFc_\epsilon R\alpha 4_{197}$.
7. The invention of Claims 1, 2, 3 or 4, wherein said $Fc_\epsilon R$ molecule is encoded by a nucleic acid molecule selected from the group consisting of $ncFc_\epsilon R\alpha 1_{609}$, $ncFc_\epsilon R\alpha 1_{591}$, $ncFc_\epsilon R\alpha 2_{609}$, $ncFc_\epsilon R\alpha 2_{591}$, $ncFc_\epsilon R\alpha 3_{617}$, $ncFc_\epsilon R\alpha 3_{597}$, $ncFc_\epsilon R\alpha 4_{591}$, $ncFc_\epsilon R\alpha 4_{687}$, $ncFc_\epsilon R\alpha 4_{991}$ and $ncFc_\epsilon R\alpha 4_{759}$.

8. The invention of Claims 1, 2, 3 or 4, wherein said $Fc_\epsilon R$ molecule is encoded by a nucleic acid molecule selected from the group consisting of: a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:19, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:27 and SEQ ID NO:30, and a nucleic acid molecule comprising an allelic variant of a nucleic acid molecule comprising any of said nucleic acid sequences.

9. The invention of Claims 1, 2, 3 or 4, wherein said $Fc_\epsilon R$ molecule is conjugated to a detectable marker.

10. The invention of Claims 1, 2, 3 or 4, wherein said $Fc_\epsilon R$ molecule is conjugated to a detectable marker selected from the group consisting of a radioactive label, a fluorescent label, a chemiluminescent label, a chromophoric label and a ligand.

11. The invention of Claims 1 or 3, wherein said putative canine IgE-containing composition comprises a bodily fluid selected from the group consisting of serum, blood and plasma.

12. The method of Claim 1 further comprising the step selected from the group consisting of: binding said canine $Fc_\epsilon R$ molecule to a substrate prior to performing step (a) to form a $Fc_\epsilon R$ molecule-coated substrate; and binding said putative IgE-containing composition to a substrate prior to performing step (a) to form a putative IgE-containing composition-coated substrate, wherein said substrate to be bound to said composition is selected from the group consisting of a non-coated substrate, an antigen-coated substrate and an anti-IgE antibody-coated substrate.

13. The method of Claim 12, wherein said antigen is selected from the group consisting of an allergen and a parasitic antigen.

14. The invention of Claims 13 or 30, wherein said allergen is derived from material selected from the group consisting of fungi, trees, weeds, shrubs, grasses, wheat, corn, soybean, rice, eggs, milk, cheese, bovine, poultry, swine, sheep, yeast, fleas, flies, mosquitos, mites, midges, biting gnats, lice, bees, wasps, ants, true bugs and ticks.

15. The invention of Claims 3 or 4, wherein said flea allergen is a flea saliva antigen.

16. The invention of Claims 3 or 4, wherein said flea allergen is selected from the group consisting of flea saliva products and flea saliva proteins.

17. The method of Claim 12, further comprising removing non-bound material from said antigen-coated substrate or said antibody-coated substrate under
5 conditions that retain antigen or antibody binding to said substrate.

18. The method of Claim 12 or 33, wherein said substrate comprises an ELISA plate, a dipstick, a radioimmunoassay plate, agarose beads, plastic beads, latex beads, immunoblot membranes and immunoblot papers.

19. The invention of Claims 12 or 32, wherein said substrate is latex beads.

10 20. The method of Claim 1, wherein said step of detecting comprises performing assays selected from the group consisting of enzyme-linked immunoassays, radioimmunoassays, immunoprecipitations, fluorescence immunoassays, chemiluminescent assay, immunoblot assays, lateral flow assays, agglutination assays and particulate-based assays.

15 21. The method of Claim 1, wherein said step of detecting comprises:

- (a) contacting said canine $Fc_\epsilon R$ molecule:IgE complex with an indicator molecule that binds selectively to said $Fc_\epsilon R$ molecule:IgE complex;
- (b) removing substantially all of said indicator molecule that does not selectively bind to $Fc_\epsilon R$ molecule:IgE complex; and
- 20 (c) detecting said indicator molecule, wherein presence of said indicator molecule is indicative of the presence of IgE.

22. The method of Claim 21, wherein said indicator molecule comprises a compound selected from the group consisting of a $Fc_\epsilon R$ molecule, an antigen, an antibody and a lectin.

25 23. The method of Claim 1, said method comprising the steps of:

- (a) immobilizing said canine $Fc_\epsilon R$ molecule on a substrate;
- (b) contacting said canine $Fc_\epsilon R$ molecule with said putative IgE-containing composition under conditions suitable for formation of a $Fc_\epsilon R$ molecule:IgE complex bound to said substrate;
- 30 (c) removing non-bound material from said substrate under conditions that retain $Fc_\epsilon R$ molecule:IgE complex binding to said substrate; and

(d) detecting the presence of said $Fc_\epsilon R$ molecule:IgE complex.

24. The method of Claim 23, wherein the presence of said $Fc_\epsilon R$ molecule:IgE complex is detected by contacting said $Fc_\epsilon R$ molecule:IgE complex with a compound selected from the group consisting of an antigen and an antibody that binds selectively to
5 IgE.

25. The method of Claim 24, wherein said compound comprises a detectable marker.

26. The method of Claim 1, said method comprising the steps of:

(a) immobilizing a desired antigen on a substrate;
10 (b) contacting said antigen with said putative IgE-containing composition under conditions suitable for formation of an antigen:IgE complex bound to said substrate;

(c) removing non-bound material from said substrate under conditions that retain antigen:IgE complex binding to said substrate; and
15 (d) detecting the presence of said antigen:IgE complex by contacting said antigen:IgE complex with said canine $Fc_\epsilon R$ molecule.

27. The method of Claim 1, said method comprising the steps of:

(a) immobilizing an antibody that binds selectively to IgE on a substrate;
20 (b) contacting said antibody with said putative IgE-containing composition under conditions suitable for formation of an antibody:IgE complex bound to said substrate;

(c) removing non-bound material from said substrate under conditions that retain antibody:IgE complex binding to said substrate; and
25 (d) detecting the presence of said antibody:IgE complex by contacting said antibody:IgE complex with said canine $Fc_\epsilon R$ molecule.

28. The method of Claim 1, said method comprising the steps of:

(a) immobilizing said putative IgE-containing composition on a substrate;

(b) contacting said composition with said canine $Fc_\epsilon R$ molecule under conditions suitable for formation of a $Fc_\epsilon R$ molecule:IgE complex bound to said substrate;

(c) removing non-bound material from said substrate under
5 conditions that retain $Fc_\epsilon R$ molecule:IgE complex binding to said substrate; and

(d) detecting the presence of said $Fc_\epsilon R$ molecule:IgE complex.

29. The method of Claim 28, wherein said canine $Fc_\epsilon R$ molecule comprises a detectable marker.

30. The kit of Claim 2, wherein said detection means further comprises an
10 antigen selected from the group consisting of an allergen and a parasite antigen, wherein said antigen induces IgE antibody production in canids.

31. The kit of Claim 2, wherein said detection means comprises an antibody that selectively binds to canine IgE.

32. The kit of Claim 2, wherein said detection means detects said canine
15 $Fc_\epsilon R$ molecule.

33. The kit of Claim 30, wherein said antigen is immobilized on a substrate.

34. The kit of Claim 30, wherein said parasite antigen is a heartworm antigen.

35. The kit of Claim 2 further comprising an apparatus comprising:

(a) a support structure defining a flow path;
20 (b) a labeling reagent comprising a bead conjugated to said antigen, wherein said labeling reagent is impregnated within the support structure in a labeling zone; and

(c) a capture reagent comprising said $Fc_\epsilon R$ molecule, wherein said capture reagent is located downstream of said labeling reagent within a capture zone
25 fluidly connected to said labeling zone in such a manner that said labeling reagent can flow from said labeling zone into said capture zone.

36. The kit of Claim 35, wherein said apparatus further comprises a sample receiving zone located along said flow path.

37. The kit of Claim 35, wherein said apparatus further comprises an
30 absorbent located at the end of said flow path.

38. The kit of Claim 36, wherein said sample receiving zone is located upstream of said labeling reagent.

39. The kit of Claim 35, wherein said bead comprises a latex bead.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/06774

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 G01N33/566 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 G01N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	WO 97 20859 A (IDEXX LAB INC) 12 June 1997 see claims 6, 7, 10-14, 16-27, 29, 30 see page 1, line 13 - line 21 see page 6, line 15 - line 16 see page 15, line 3 - line 16 see page 29, line 12 - line 25 ---	1-39
X	WO 95 16203 A (GENENTECH INC; TAI WAI FEI DAVID (US); LOWE JOHN (US); JARDIEU PAU) 15 June 1995 see claims 1, 8, 14-16 see page 4, line 18 - page 6, line 9 see page 12, line 25 - page 13, line 5 see page 13, line 14 - line 19 see page 23, line 9 - page 24, line 38 --- -/--	1-39



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

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"Z" document member of the same patent family

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INTERNATIONAL SEARCH REPORT

Inter onal Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

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